DISSERTATION ON

STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the regulations

for the award of the degree of

M.D. DEGREE IN GENERAL MEDICINE

BRANCH – I

THANJAVUR MEDICAL COLLEGE,

THANJAVUR - 613 004

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

APRIL – 2013

CERTIFICATE

This is to certify that this dissertation entitled " **STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA**" is the bonafide original work of **Dr. MOHAMMED NIAZ .A.A** in partial fulfilment of the requirements for **M.D. Branch – I (General Medicine)** Examination of the Tamilnadu Dr.M.G.R. Medical University to be held in APRIL - 2013. The period of the study was from April – 2012 to November -2012.

Prof.Dr.S.MUTHUKUMARAN, M.D.,
Unit Chief M-1
Dept. Of Internal Medicine,
Thanjavur Medical College,
Thanjavur – 613004.

Prof.Dr.S.MUTHUKUMARAN,M.D.,
Head Of the Department,
Dept. Of Internal Medicine,
Thanjavur Medical College,
Thanjavur – 613004.

Prof. Dr. C. GUNASEKARAN, M.D., D.C.H.,
DEAN in charge,
Thanjavur Medical College,
Thanjavur – 613 004.

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA-613004

(Affiliated to the T.N Dr.MGR Medical University, Chennai)

ETHICAL COMMITTEE

CERTIFICATE

Name of the Candidate : **Dr. MOHAMMED NIAZ .A.A**

Course : **M.D., GENERAL MEDICINE**

Period of Study : **April -2012 to November -2012**

College : **THANJAVUR MEDICAL COLLEGE**

Dissertation Topic : **STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA**

The Ethical Committee, Thanjavur Medical College has decided to inform that your Dissertation Topic is accepted and you are permitted to proceed with the above study.

Thanjavur Secretary

Date : Ethical Committee

DECLARATION

I, **Dr. MOHAMMED NIAZ .A.A**, solemnly declare that dissertation titled " **STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA** " is a bonafide work done by me at Thanjavur Medical College, Thanjavur during April - 2012 to November - 2012 under the guidance and supervision of **Prof. Dr. S. Muthukumaran, M.D.,** Unit Chief M-I, Department of Internal Medicine, Thanjavur Medical College, Thanjavur.

This dissertation is submitted to Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of **M.D. Degree (Branch - I) in General Medicine**.

Place: Thanjavur,
Date: 20-12-2012.

(**Dr. MOHAMMED NIAZ .A.A**)
Postgraduate Student,
M.D. in General Medicine,
Thanjavur Medical College,
Thanjavur - 613 004.

ACKNOWLEDGEMENT

I gratefully acknowledge and express my sincere thanks to **Prof.Dr.C.GUNASEKARAN, M.D.,D.C.H.,** Dean in charge, Thanjavur Medical College, Thanjavur for allowing me to do this dissertation and utilize the Institutional facilities. I am extremely grateful to **Prof.Dr.S.Muthukumaran, M.D.,** Head of Department, Department of Internal Medicine, Thanjavur Medical College for his full-fledged support throughout my study and valuable suggestions and guidance during my study and my post graduate period.

I wish to express my deep sense of gratitude and respect to my beloved teacher and mentor **Prof.Dr.S.Muthukumaran, M.D.,** my Professor and Unit Chief, who is my guide in this study, for his timely suggestions, constant encouragement and scholarly guidance in my study and Post Graduate period.

I profoundly thank my respected professors **Prof. Dr. P.G. Sankaranarayanan, M.D., Prof. Dr. K. Nagarajan, M.D., Prof. Dr. S. Manoharan, M.D., Prof. Dr. C. Ganesan, M.D., Prof. Dr. D. Nehru, M.D., D.M.R.D.,** and **Dr. C. Paranthakan, M.D.,** Registrar for their advice and valuable criticism which enabled me to do this work effectively.

I would like to express my gratitude to **Prof. Dr. T. Rajendran, M.D., DM (Nephro).,** Associate Professor and Head, Department of Nephrology for his immense help in the study which enabled me to complete this work.

My grateful thanks to **Prof. Dr. N. SASIVATHANAM, M.D.,D.G.O.,** Head and Professor of Biochemistry, for her valuable guidance. I am grateful for the help rendered by the technicians of the Department of Biochemistry in the preparation of this study.

My sincere thanks to Assistant Professors **Dr. C. Paranthakan, M.D.,** and **Dr. V. Ravichandran, M.D.,** for their motivation, encouragement and support.

I am indebted to my parents, all my friends, colleagues, and my wife Raiza**,** for their constant encouragement throughout this study.

Last but not least, I am grateful to all those patients who were the subjects for the study, without whose co-operation this work would not have been possible.

turnitin

Your digital receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

Paper ID	291081168
Paper title	STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA
Assignment title	Medical
Author	Mohammed Niaz 20101177 M.D. General Medicine
E-mail	getniaz4ever@yahoo.co.in
Submission time	21-Dec-2012 10:56AM
Total words	8551

First 100 words of your submission

DISSERTATION ON STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA Dissertation submitted to THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY In partial fulfilment of the regulations for the award of the degree of M.D. DEGREE IN GENERAL MEDICINE BRANCH – I THANJAVUR MEDICAL COLLEGE, THANJAVUR - 613 004 THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI - 600 032 APRIL – 2013 1 INTRODUCTION Increased protein in the urine is a common sign of kidney disease. A urine sample can be easily obtained usually, and it can be analyzed by very simple techniques in the ward or in the out-patient setup. Analysis of urine is one of the most rewarding tests in clinical...

DISSERTATION ON

STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

...al fulfilment of the regulations

... the award of the degree of

...REE IN GENERAL MEDICINE

BRANCH – I

STUDY OF SPOT URINE PROTEIN CREATININE RATIO AS AN INDEX OF QUANTITATIVE PROTEINURIA

ABSTRACT

Back Ground:

Quantitating protein in the urine has become more than just a diagnostic test. It can be even used for prognostic purposes and to assess the effect of therapy. Commonly used method to measure protein is 24 hours urine collection, which is time consuming, cumbersome and often inaccurate.

Aim :

Aim of the study was to compare spot urine protein- creatinine ratio with 24 hours urine protein as an index of quantitative proteinuria.

Method:

68 patients with proteinuria with varying degrees of renal dysfunction were included in this study. 24 hour urine protein estimation was done and protein creatinine ratio was calculated from a spot urine sample and compared. Study group was divided into 4 groups according to creatinine clearance and degree of proteinuria. Correlation between 24 hours urine protein and spot urine protein creatinine ratio, among the groups was statistically analyzed.

Results:

There was significant correlation between 24 hours urine protein and spot urine protein-Creatinine ratio ($r = 0.975$) ($P < 0.05$). However least correlation was in patients with end stage renal disease having nephrotic range proteinuria ($r = 0.868$) ($P < 0.05$).

Conclusion :

Spot urine protein creatinine ratio is a useful index for quantification of proteinuria, which is easy to perform, inexpensive and less time consuming method.

Key words: spot urine Protein-Creatinine ratio, 24 hour urine protein, Proteinuria, creatinine clearance

CONTENTS

INTRODUCTION

Increased protein in the urine is a common sign of kidney disease. A urine sample can be easily obtained usually, and it can be analyzed by very simple techniques in the ward or in the out-patient setup. Analysis of urine is one of the most rewarding tests in clinical medicine, as not only does it uncover renal diseases but also frequently points to a specific diagnosis. Testing the urine for proteinuria has been part of the routine clinical examination for nearly 200 years [1].

Quantitating protein in the urine has become more than just a diagnostic test. Even a relatively small increase in the concentration of protein in the urine can be an early sign of renal disease and often precede a detectable change in glomerular filtration rate (GFR).

Protein in the urine is more than a simple marker of disease, as persistently high levels may cause further kidney damage and result in faster progression of the kidney disease. In addition, proteinuria is an independent and strong risk factor for cardiovascular disease and death, mostly in people with diabetes, hypertension and chronic kidney disease. Interventions that reduce the

amount of proteinuria also tend to retard the progression of kidney disease and improve the prognosis of cardiovascular disease.

Proteinuria has become a common presentation of renal disease since sticks testing for protein became widely available, and there is frequent screening of apparently healthy individual. Protein, blood or both can be found in the urine of apparently normal individual. Vigorous exercise, fever or UTI, and heart failure also can cause transient mild albuminuria. In such cases, tests should be done again when the trigger is no more present. There is still much controversy about who should have their urine tested and how anyone found to have positive test should be further investigated and managed. The prevalence of proteinuria on a routine screening of healthy subjects has been found to be as high as 3.5%. It is important that all patients with proteinuria be carefully evaluated to identify the cause of proteinuria.

Normal daily protein excretion in an adult does not exceed 150 mgs. Persistent proteinuria of > 1.0 gm/day, usually indicates renal disease. Proteinuria may be minimal (<1.0 gm/day), moderate (1–3 gm/day) and heavy (>3 gm/day). Important causes of minimal proteinuria are chronic pyelonephritis, diabetic nephropathy, interstitial nephritis and chronic renal

failure. Moderate proteinuria is seen in nephritic syndrome and toxic nephropathies and heavy proteinuria indicates active glomerulonephritis. So quantification of protein is very important.

Current methods for measuring proteinuria vary significantly. Commonly used methods are dipstick urine analysis, 24 hrs urine protein estimation and spot urine protein Creatinine ratio.

Only very few Indian studies have compared the efficacy of 24 hours urine protein with spot urine protein creatinine ratio, which this study attempts to do.

AIMS AND OBJECTIVES

1. To assess quantitative measurement of proteinuria by using spot urine protein creatinine ratio

2. To compare spot urine protein creatinine ratio with 24 hours urine protein as an index of quantitative proteinuria.

3. To determine the correlation of spot urine protein creatinine ratio with 24 hour urine protein at different levels of glomerular filtration rates (GFR).

REVIEW OF LITERATURE

Proteinuria is a frequent finding in primary care practice. Hippocrates was the first one to note the relation between "Bubble on the surface of urine" and renal disease 2400 years ago [6, 7].

Moderate amount of low molecular weight proteins pass through the healthy glomerular basement membrane. Such proteins are normally reabsorbed by receptors on tubular cells. Normal individuals excrete <150 mg of total protein and <30 mg of albumin in urine every 24 hours. Other proteins found in the urine are either secreted by tubules (Tamm-Horsfall protein) or are small filtered proteins that have escaped reabsorption or degradation by renal tubule cells. Any level of protein excretion in the urine above 150 mg per 24 hours is abnormal and merits further evaluation.

Under normal conditions, filtered proteins from plasma make half of this small amount of urinary protein and the rest is from the proteins which are secreted into the urine from urinary tract cells. These filtered proteins include small amounts of albumin (approximately 15% of the total urinary protein), immunoglobulins (5%), light chains (5%), β2 microglobulin (less than 0.3 %), and other plasma proteins (25%). The most prevalent tubular protein (and the most

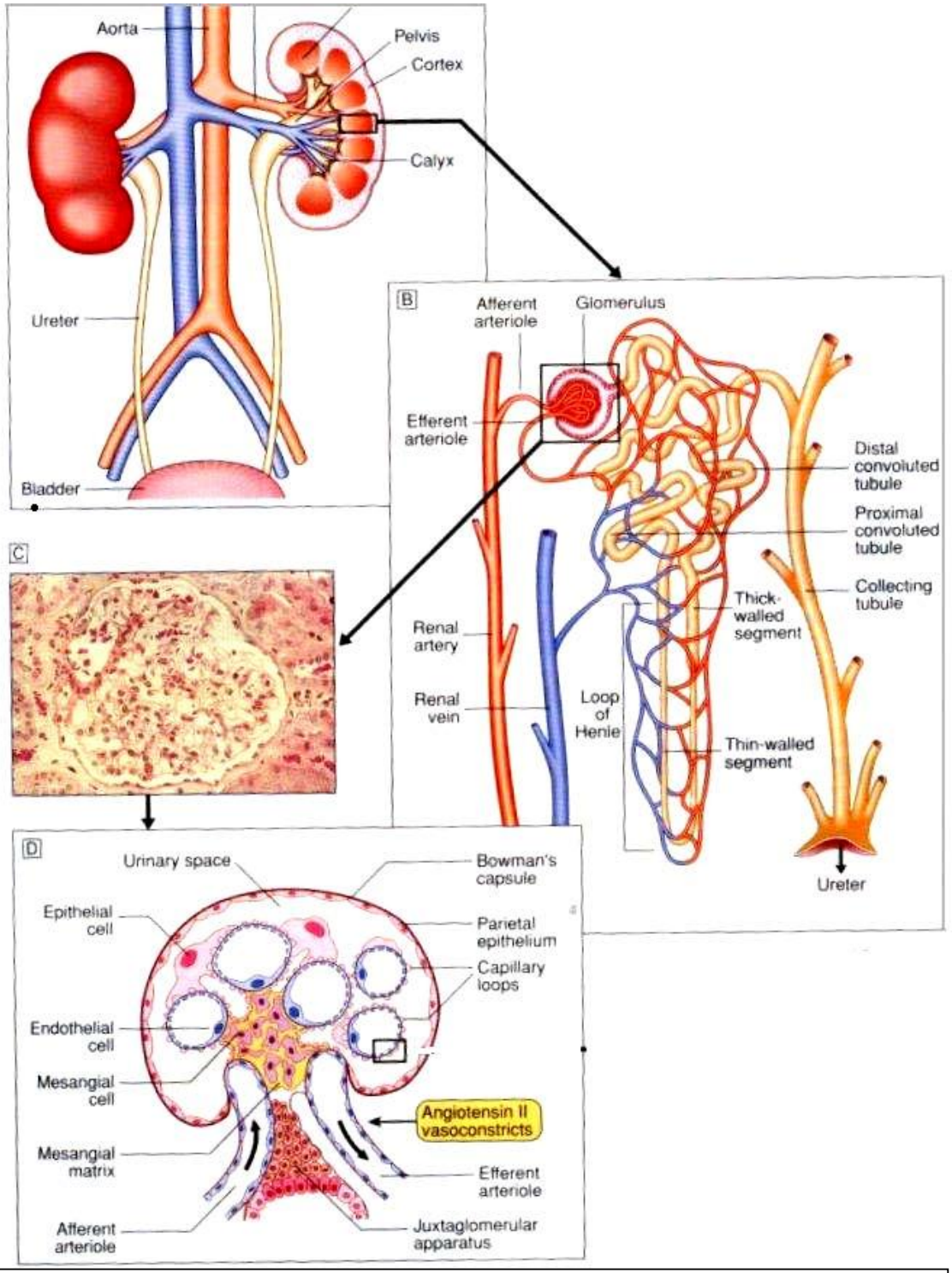
abundant protein in normal urine) is Tamm-Horsfall protein. It is synthesised in the tubular cells of the ascending limb of the loop of Henle and is secreted into the urine. There is no noticeable sign of proteinuria unless the excretion is greater than several grams per 24 hours. In that case the urine may become frothy because proteins lowers the surface tension of the urine and permits relatively stable foam to form [1]. This frothing may be a valuable way of dating the onset of profuse proteinuria.

Protein handling by normal kidney

The normal glomerular endothelial cells form a barrier and hold back cells and other particles. They are penetrated by large pores of 100nm called fenestrae that can easily be traversed by proteins. The glomerular basement membrane traps most large proteins (>100Kda). The urinary side of the glomerular basement membrane is covered by the foot process of epithelial cells (Podocytes). It produces a series of narrow channels (Slit diaphragm) to allow passage of small solutes and water. This slit diaphragm bridges the slits between the foot processes of the glomerular basement membrane [8]. Negatively charged heparan sulfate proteoglycans cover the visceral epithelial cells [6]. This negative charge and size selectivity of glomerular basement

membrane impedes the passage of anion molecules such as albumin, globulin and large molecular weight protein across the glomerular wall. The smaller proteins that are filtered across the glomerular basement membrane are largely reabsorbed at the proximal tubule and only small amount are excreted.

FIGURE 1 : <u>FUNCTIONAL ANATOMY OF KIDNEY</u> [25]



A: Anatomical relations of kidney; B: Single nephron ; C: Histology of normal glomerulus ; D: Cross section of glomerulus

FIGURE 2 : ELECTRON MICROSCOPIC PICTURE OF NORMAL GLOMERULAR BASEMENT MEMBRANE

.

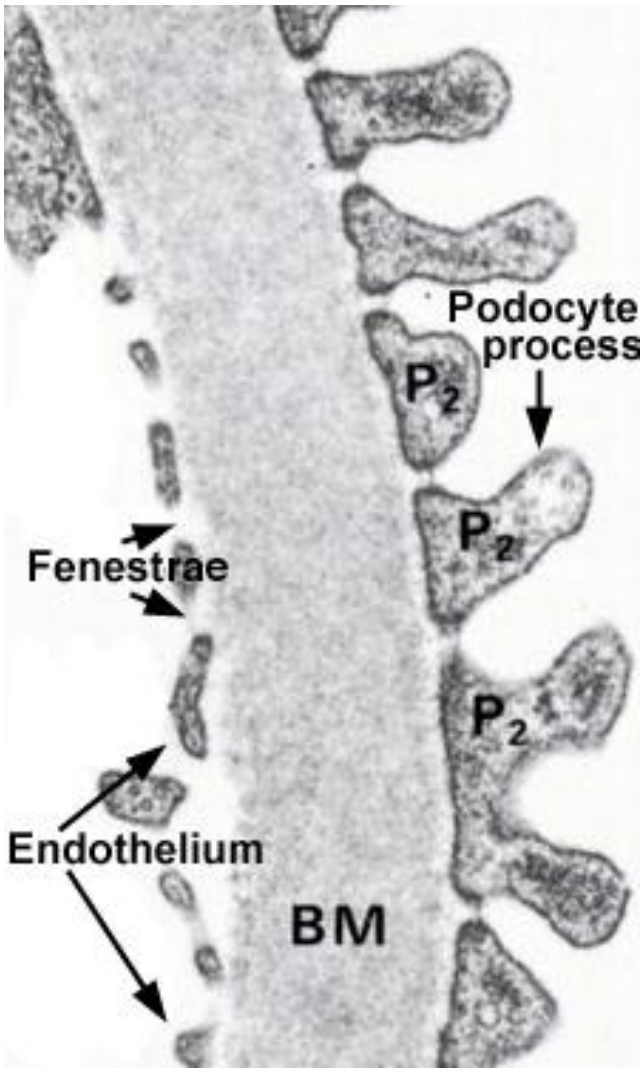


FIGURE 3 : NORMAL PODOCYTE STRUCTURE

PATHOPHYSIOLOGICAL CLASSIFICATION OF PROTEINURIA

A) BENIGN

1. Postural / Orthostatic proteinuria
2. Functional
3. Transient idiopathic
4. Intermittent

B) PATHOLOGICAL

1. Glomerular
2. Tubular
3. Overflow
4. Secretory

A) BENIGN PROTEINURIA

This is a transient proteinuria that occurs with normal renal function, bland urinary sediment, normal blood pressure and without any significant edema. 24 hour urine protein is usually less than 1 gm. They do not indicate any significant renal disease and disappears on repeated testing.

1) POSTURAL / ORTHOSTATIC PROTEINURIA

Benign orthostatic proteinuria is mostly seen in tall and young individuals, and occurs when the patient is in the lordotic position. It is diagnosed by split urine protein excretion examination. Urine specimen collected during the day time shows raised amount of protein, but not in the overnight sample collected immediately on waking up. Urine sediment does not show any abnormality, and proteinuria will be less than 1 gram/day. In most of the patients, proteinuria will disappear within ten years; however, in a small portion, overt renal disease may develop later.

Springberg et al [11] found that long term prognosis of orthostatic proteinuria is benign in virtually all cases over many decades. Data on renal biopsies on orthostatic proteinuria are confusing. Some showed minor glomerular changes [12]. Posture affects urinary protein excretion, probably via an increase in glomerular capillary hydrostatic pressure and change in permeability of the glomerular capillary walls[13]. An alternate explanation is entrapment of renal veins [14 15].

2) FUNCTIONAL

It is a benign proteinuria due to changes in glomerular ultra filtration pressure and / or membrane permeability. It is seen in Fever, Exercise, Cardiac failure, Emotional stress and acute illness. It is usually less than 0.5 gm/day but may be as heavy as 5.0 gm/day (following marathon running). It disappears with the resolution of causative disorder [16].

Kallmeyer et al [17] found that recent exercise can induce several gram of protein per liter of urine, sometimes together with haematuria and even casts. So called jogger's nephritis. Post exercise proteinuria is about 15 to 20 times the resting range of proteinuria and require about 4 hours to regain resting value in the recovery period [18]. **Poortmans et al** [19] found that proteinuria was influenced mostly by the intensity of exercise rather than its duration.

3) IDIOPATHIC PROTEINURIA

This is seen in young healthy adults. This dipstick positive proteinuria disappears spontaneously by next clinical visit.

4) INTERMITTANT PROTEINURIA

This benign proteinuria is found in half of their different urine samples in absence of other renal or systemic abnormalities.

B) **PATHOLOGICAL PROTEINURIA**

This is persistent proteinuria that is detected on multiple ambulatory clinical visits. This is seen in both recumbent and upright position and usually signals a structural renal disease.

1) GLOMERULAR PROTEINURIA

Glomerular proteinuria takes place when injury to the glomerulus lead to an ultra filtrate which constitutes partial rise in the clearance of serum proteins. This type of proteinuria is peculiarized by an excessive amount of albumin in urine [20]. Due to preservation of selectivity and large concentration of albumin in blood, glomerular proteinuria is 85 to 90 % albumin, accompanied by pre- albumin, orosomucoid, transferrin and relatively low molecular weight proteins. They are readily detected by stick or turbidimetric methods. Glomerular proteinuria is often classified either as non nephrotic (< 3.5 grams/ day/1.73 m^2 BSA) or nephrotic (>3.5 grams/day/1.73 m^2 BSA). **McConnell et al** on evaluation of proteinuria found that urinary excretion of > 2 gm / day is usually a result of glomerular disease [21]. In glomerular proteinuria, there is increased glomerular capillary permeability to high molecular weight anionic plasma proteins. How the glomerular barrier is damaged so that it leaks more than normal remains unclear [22]. This may be due to :

- Loss of fixed anionic charge (congenital nephrotic syndrome, minimal change nephropathy)
- Detachment of epithelial podocytes from basement membrane [23.]
- Immune aggregates
- Increase in glomerular capillary pressure.

The filtered proteins, that reach the tubules overwhelm the limited capacity of tubular reabsorption and cause these proteins to appear in urine.

Glomerular disease is classified as primary when the pathology is confined to the kidney and secondary when it is a part of multi system disorder.

Glomerular proteinuria is of two types:

- Selective Proteinuria
- Nonselective Proteinuria

In selective proteinuria the clearance ratio of immunoglobulin to albumin or transferrin is less than .10 (<10%). In nonselective proteinuria the clearance ratio of immunoglobulin to albumin or transferrin is more than 0.50 (>50%).

GLOMERULAR PROTEINURIA - CAUSES :

- Primary glomerular disorders

  Minimal change, FSGS, membranoproliferative, mesangial proliferative, membranous, crescentic

- Hereditary

  Alport's syndrome, nail – patella syndrome , Fabry's disease

- Infectious

  Bacterial, fungal, protozoal, viral, and helminthic causes, including bacterial endocarditis, hepatitis B and C, visceral abscesses, PSGN, secondary syphilis, malaria , HIV

- Metabolic

  DM

- Immunologic

  SLE, MCTD, Sjogren's syndrome, HSP, Wegener granulomatosis, microscopic PAN, Goodpasture's syndrome, cryoglobulinemia

- Medications

  lithium, NSAIDs, Penicillamine, ACE inhibitors, heroin, gold or mercury containing compounds

- Neoplasms

lymphoma , Multiple myeloma, leukemia,

colon, lung, or breast carcinoma

- Miscellaneous

congenital nephrotic syndrome , Sickle cell disease, reflux nephropathy, cirrhosis, immunotactoid glomerulopathy, amyloidosis, allergies, immunizations

2) TUBULAR PROTEINURIA

Proteinuria results from the damage of proximal tubule so that normally reabsorbed protein, principally of low molecular weight, pass into the urine .This usually occurs as a part of the Fanconi syndrome of proximal tubular dysfunction. Tubular proteinuria usually does not exceed 2gm per day [24 25].

Tubular proteinuria is originated from 3 sources :

1. Damaged tubules incompletely reabsorb low molecular weight proteins filtered by the glomerulus, like β_2 microglobulin & amylase.

2. Damaged tubules secrete cellular enzymes & brush border components , like N-acetylglucosaamine & lysozyme, into the urine.

3. The tubular cells of the acending limb of the loop of Henle & the distal nephron may secrete Tamm-Horsfall proteins in excess amount when tubulointerstitial damage occurs.

Beta 2-Microglobulin is one of the many micro globulin which make up tubular proteinuria. Normal level of Beta 2- Microglobulin in urine is less than 0.4 mcg/L. It can be assessed by RIA or ELISA. The urinary albumin - β_2 microglobulin ratio of 10:1 indicates tubular proteinuria. In glomerular proteinuria, this ratio will be more than 1,000:1 [3]. In normal conditions, albumin - β_2 microglobulin ratio ranges between 50:1 and 200;1. Further measurement of Beta 2-M Lysozyme may help in distinguishing type of urinary tract infection besides diagnosis of heavy metal poisoning [26 27]. Urinary protein electrophoresis, Immuno electrophoresis, and immunofixation also may aid in distinguishing tubular and glomerular proteinuria.

TUBULAR PROTEINURIA – CAUSES [28]

-Hypertensive nephrosclerosis

-Tubulo interstitial diseases due to

-Fanconi syndrome

-Heavy metals

-Uric acid nephropathy

-Acute hypersensitivity

-Interstitial nephritis

-Sickle cell disease

-Drugs (NSAID, Antibiotics)

3) OVERFLOW PROTEINURIA

When a low molecular weight protein is produced in abnormally excessive amount in the body, it will be filtered through the normal glomerulus and the normal tubules may fail to reabsorb it completely, as it exceeds its normal capacity to reabsorb. This is known as overflow proteinuria. This happens in monoclonal gammopathies like multiple myeloma, in intravascular hemolysis (hemoglobinuria), & in rhabdomyolysis (myoglobinuria). It may be recognized by the presence of aberrant spike in urinary electrophoresis. [28 29]

OVERFLOW PROTEINURIA –CAUSES

- Multiple myeloma
- Hemoglobinuria
- Myoglobinuria

- Rhabdomyolysis
- Lymphoproliferative disorders

4) SECRETORY PROTEINURIA

It occurs due to secretion of proteins into the urine after glomerular filtration. About 20 to 30 mg/24 hours of non plasma protein is contributed by renal tubules and lower urinary tract**.** Mostly they are formed by Tamm-Horsfall proteins[29.] Some secretary IgA is added by lower urinary tract including the urethral glands together with trace quantity of protein of prostatic or seminal vesicular organ [30 31].

Tamm-Horsfall protein is secreted by the ascending thick limb and early distal convoluted tubule into the tubular fluid. It is an easily polymerized glycoprotein . They form the major constituent of renal tubular casts [32], along with albumin and traces of many plasma proteins, including immunoglobulins [33]. In Myeloma, casts contain paraproteins polymerized with Tamm-Horsfall protein, and may show a micro fibrillar structure that will stain positive with congo red, even though no amyloid is present in renal tissue.

Table 1: DEGREE OF PROTEINURIA AND CAUSES [20]

Daily protein excretion	Causes
0.15 – 2.0 gm	Mild glomerulopathies Tubular proteinuria Overflow proteinuria
2.0 –3.5 gm	Usually Glomerular
>3.5 gm	Always glomerular

MICROALBUMINURIA

It denotes urinary albumin excretion of 30 to 300 mg per day or 30 to 300 mg/gram creatinine in a spot collection [34]. Conditions like essential hypertension and diabetes may manifest as microalbuminuria. It is established that those with microalbuminuria have increased risk of developing overt diabetic nephropathy, renal failure and cardiovascular complications in future. The exact mechanism of micro albuminuria is not understood well. The explanation to the question why it is associated with these conditions is yet to be established. It may be the earliest clinical manifestations of

involvement of kidney in diabetes mellitus.

Microalbuminuria is the most reliable indicator of incipient diabetic nephropathy within the first 10 years of type 1 diabetes, when the majority of patients with microalbuminuria will progress to overt nephropathy within a further 10 years. It is a less reliable predictor of nephropathy in older patients with type 2 diabetes. The prevalence of microalbuminuria rises to reach 50 - 60% after 20-30 years duration of diabetes [35.] Standard dipsticks for protein fail to detect microalbuminuria. It can be measured only by sensitive methods. It can be tested by immunometric dipstick micral test. Screening is conducted yearly in patients with type 2 diabetes, but can be deferred for the first 5 years in those with type 1 diabetes[38].

METHODS OF DETECTING AND MEASURING PROTEINURIA

A) DETECTION OF PROTEINURIA

1 Dipstick analysis

2 Precipitation methods

B) QUANTIFICATION OF PROTEINURIA

1. Turbidimetric method
    - Sulphosalycilic acid
    - Benzethonium chloride
    - Trichloroacetic acid
2. Biuret method
    - Copper reagent
    - Tsuchiya reagent
    - Fowlin-Lowry
3. Dye binding technique
    - Pyrogallol red
    - Coomassie brilliant blue
    - Ponceau S
4. Radio immune assay
5. Enzyme linked immunosorbent assay

C) CHARECTERIZATION OF PROTEINURIA

1. Immune electrophoresis
2. Column gel chromatography
3. Agarose gel electrophoresis
4. Polyacrylamide gel electrophoresis
5. Isoelectric Focussing

A) DETECTION OF PROTEINURIA

1) DIPSTICK ANALYSIS

It is one of the most commonly used method to detect proteinuria in out patient settings. It semi quantitatively measures the urine protein concentration. Tetrabromophenol is buffered to a pH of 3, and with rising protein concentration, a yellow – to – green colour change can be observed .

It preferentially detect negatively charged urinar proteins (albumin). The lower threshold concentration for detection of protein by the routine dipstick is around 15 to 20 mg/dl which is roughly equivalent to a 24 hour urine protein of 300 to 500 mg [2]. Light chains and some low molecular weight protein are not detected by stick tests. The sticks are buffered to keep the pH constant. Leaving

the sticks in the urine will wash out the buffer and give a false reading. They should be read immediately.

Ralston et al [39] found that dipstick testing though nearly 100% sensitive, has a poor specificity due to high false positive rates. Specificity being 40% with 1 + , 83% with 2 + and 48% with 3 + readings.

Scale for detecting proteinuria on routine urine dipstick:

Negative = < 15 mg/dl

Trace = 15 to 30 mg/dl

+ = 30 to 100 mg/dl

++ = 100 to 300 mg/dl

++ + = 300 to 1000 mg/dl

++++ = more than 1gram/dl

David son et al [40] while evaluating the relation between dipstick positive proteinuria and albumin-creatinine ratio found that dipstick positive proteinuria of ≥ 1 + may be used as a substitute for albumin - Creatinine ratio in random urine specimen for proteinuria quantification. Presently, the American diabetic association and the Kidney foundation do not

consider dipstick urine test for protein for evaluation of diabetic nephropathy. **Meyer et al** [42] found out that sixty six percentage of patients with negative or trace protein had significant proteinuria when the specimen was compared with 24 hours urine collection.

FALSE POSITIVE DIPSTICK PROTEINURIA

- Ph > 7
- Pus
- Highly concentrated urine
- semen
- Gross Haematuria
- Dipstick immersed too long
- Drugs like Penicillin, Sulfonamide or Tolbutamide.
- Radiocontrast
- vaginal secretions

FALSE NEGATIVE DIPSTICK PROTEINURIA

- Highly dilute urine
- non albumin proteins such as immunoglobulins
- low molecular weight proteins

PRECIPITATION METHOD

Kjeldahl :-

This precipitation method measures protein by measurement of precipitated Nitrogen. Detection limit is 10-20 ng/l.

The heat test, eventhough it is valid, is not usually done nowadays. A sulphosalycilic acid precipitation test can be used instead.

QUANTIFICATION OF PROTEINURIA

1) **Turbidimetric method**:

In this, addition of Trichloro acetic acid or sulphosalicylic acid alters colloid properties and produces turbidity to be read in densitometer. Occasionally Benzethonium is also used instead of sulphosalycilic acid. Its detection limit is 50-100 mg/l. The disadvantage of this method is that it is imprecise and gives different readings for albumin and globulin. The advantage of this test is that it can be easily performed and it has a greater sensitivity for proteins like Bence Jones. A positive sulphosalycilic acid test result with a normal dipstick denotes the presence of non albumin proteins in the urine as might

occur with multiple myeloma. A few ml of freshly voided, centrifuged urine specimen is added with equal amount of three percentage sulphosalycilic acid. Turbidity will develop with a protein concentration of more than or equal to 4 mg/dl. False positive results may occur if patient had recent administration of radio contrasts or he/she is on drugs like Penicillin or sulfonamides [43]. A highly buffered alkaline or dilute urine sample may give a false negative report.

2) **Biuret Method:**-

This requires precipitation of protein, by using a copper or Tsuchiya reagents. Its detection limit is 50 mg/l. They are used in some laboratories to measure 24 hours urine protein.

3) **Dye binding technique**:-

This is most widely used technique for quantification of urinary proteins. It is based on the interaction between protein and a dye. They are reliable, accurate and an easy way for assessing proteinuria. Commonly used dyes are pyrogallol red, Coomassie brilliant blue and Ponceau S.

Pyrogallol red dye is the most commonly used dye for dye binding technique. They over come many of the traditional limitations associated with colorimetric proteins, as there is minimal staining of plastics and glassware. This makes it an ideal dye for most chemical analyzers. Pyrogallol red combines with sodium molybdate to form a red complex. Proteins, in an acidic medium combine with this red complex and form a bluish purple colored complex. The intensity of the color formed is directly proportional to amount of proteins present.

Proteins + Pyrogallol red + Molybdate

In acidic medium ↓

Bluish purple colored complex.

COLLECTION OF URINE

There are various methods for collection of urine. Currently acceptable methods are :[44]

1) 1-2 hour collection
2) Over night (8-12 hour) collection
3) 24 hour collection
4) Random urine sample for protein Creatinine ratio

A 24 hours urine collection is usually required for a precise quantitative and qualitative analysis of the amount and composition of urinary proteins. The urine is collected by emptying the bladder and discarding the first urine on the morning of collection, and meticulously collecting all ensuing urine for the next 24 hours. The final urine at the end of the 24 hour period is also collected.

The timed specimen (24 hour or over night) is more sensitive but the Protein- Creatinine ratio is more practical and convenient for the patient [44].

STORAGE OF SPECIMEN:

Estimation of protein from urine should be done as soon as possible after the collection. If estimation of protein is delayed by 24 hours then it should be stored at a temperature less than 18 degree centigrade. Urine can be stored at this temperature for a maximum period of 3 days. No preservative is usually required if the urine is refrigerated during the 24 hour period. A preservative like acetic acid may be added to the collection vessel if refrigeration is unaccessible. Refrigerated urine sample should be allowed to reach at least 10 degree centigrade before analysis. It is observed that freezing

samples decrease albumin, but mixing immediately before assay eliminates this effect.

ADEQUACY OF COLLECTION

Creatinine is produced from skeletal muscle Creatine, as a metabolic product, at a constant rate. The amount of creatinine produced is directly proportional to skeletal muscle mass. With an unfluctuating state of renal function, each gram of creatinine in 24hour urine collection equate 18.5gms of fat free skeletal muscle mass[45.]

As the concentration of creatinine remains relatively constant on a daily basis it can be used to assess the adequacy of timed urine collections. The urinary creatinine excretion is measured and compared with normal expected ranges of creatinine excreted per day. The normal creatinine excretion per day is as follows:

Males : 18 – 25 mg/kg/day

Female : 15-20 mg/kg/day

If expected Creatinine is similar to what has been measured in previous timed urine sample, the collection is likely to be accurate.

URINE PROTEIN CREATININE RATIO

The quantification of proteinuria is commonly used in the diagnosis, assessment and prognosis of glomerular disease [46].

Quantitative estimation of daily urinary protein excretion is usually done by 24 hours urine collections. However such timed collections of urine are inconvenient, cumbersome and at times unreliable because of frequent errors in collection and up to a third of the specimens have to be rejected [47].

A urinary protein – creatinine concentration ratio on spot urine sample can be used as a quick assessment of proteinuria, and it can be useful as an alternative for repeated full 24 hour urine collections to assess responsiveness to therapy [48] [49] .

The amount of dilution of the urine will directly affect protein concentration. The concentration of creatinine in the urine serves as an internal control for the urine dilution. The ratio of protein to creatinine is independent of urine concentration, as concentration affects both parameters equally. There is evidence that untimed or spot urine specimen is adequate for quantification of

proteinuria. Since the rate of creatinine excretion remains fairly constant, the ratio of protein to creatinine on a random specimen is a good estimate of amount of protein excreted. It is important to note the units in which the laboratory reports protein and creatinine, as equivalent units should be used to generate a valid ratio.

The protein and creatinine concentration in urine are measured by routine biochemical analyzers and the ratio is determined. Assuming that the average individual excretes approximately 1 g of creatinine per day, normal spot urine protein – creatinine ratios on random samples usually fall below 0.2 (mg protein per mg creatinine), whereas values greater than 3 suggests the presence of nephrotic range proteinuria. For instance, a ratio of 2 roughly translates to a 24 hour urine protein excretion of 2g. The preciseness of the ratio is diminished when creatinine excretion is either markedly increased in a muscular man (the ratio will underestimate proteinuria) or markedly reduced in cachectic patient (the ratio will overestimate proteinuria).

Gisberg et al [48], on quantitative estimation of proteinuria from an single void urine, found an excellent correlation between the Protein–creatinine ratio in a single urine sample and the protein content of a 24 hour urine collection. Correlation between 24 hours urine protein excretion and the urinary

Protein – Creatinine ratio has been validated in several diseases, including preeclampsia, diabetes mellitus, and rheumatic disease [39 46 50].

Silink et al [51] studied the relationship between albumin concentration in 24 hour urine and urine samples at various times of the day, in patients with type 1 diabetes mellitus. He found out that the correlation was highest with first morning urine sample. Hence the urine collected from the first morning sample for Protein- Creatinine ratio, is an acceptable alternative to a 24 hours urine collection for proteinuria quantification in clinical follow up and screening [52 53].

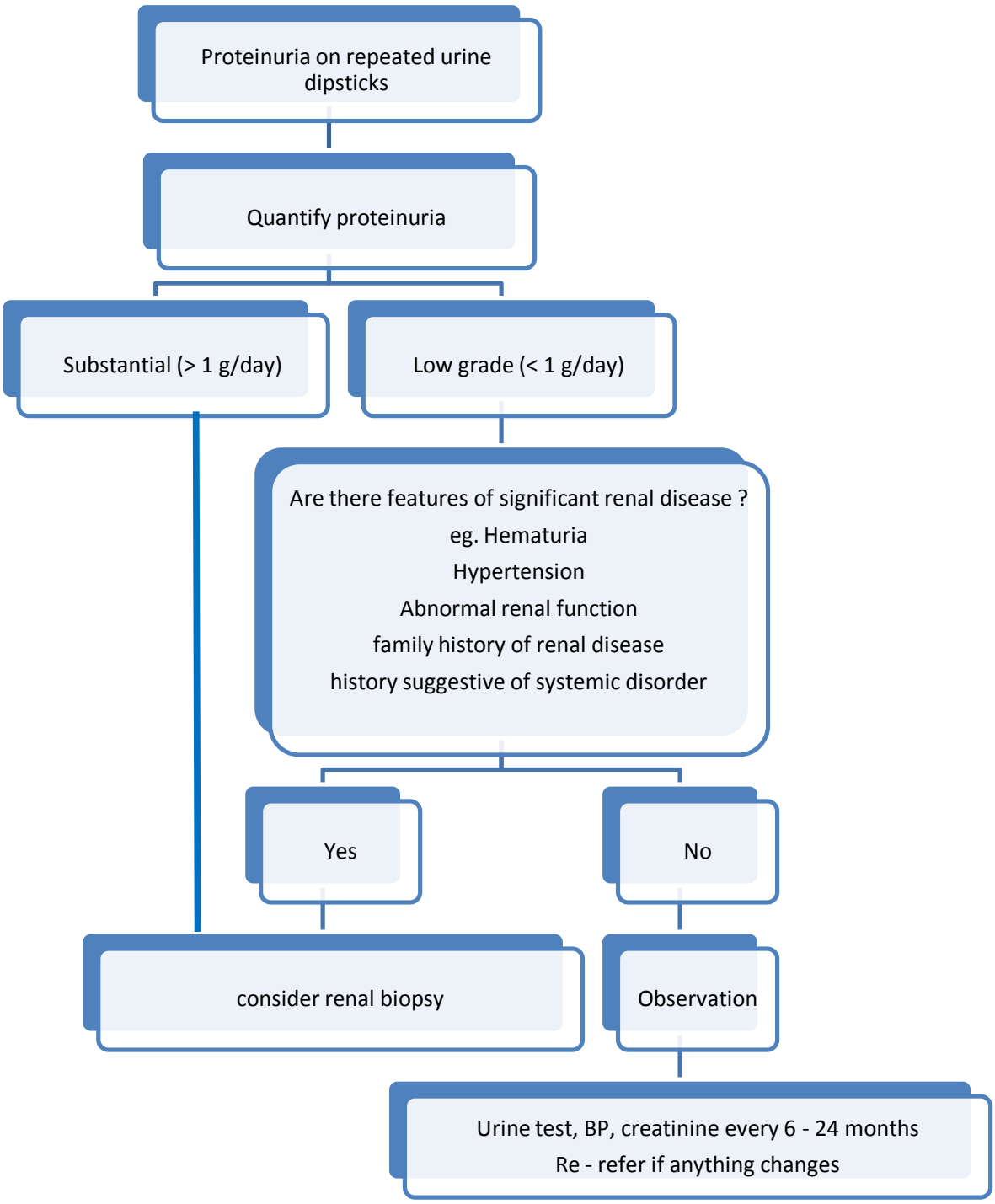
Rodby et al [46] in is study found that time of day when the random urine specimen was obtained did not impact on the ability to predict 24 hours urine protein from random urine specimen.

Another method for quantification of proteinuria in spot urine sample is by measuring urine Protein – Osmolality ratio. **Wilson et al** [54] found that urinary protein osmolality ratio indicated quantitative proteinuria with reasonable predication and is more useful than qualitative urine analysis for detecting or assessing proteinuria.

Zelmanovitz et al [55] found that proteinuria estimation in a random urine sample is a simple and reliable method for screening and diagnosis of overt diabetic nephropathy.

Ruggenenti et al [56], on study of 24 hours urine protein excretion, spot morning urine Protein-Creatinine ratio and glomerular filtration in chronic renal disease in patients without diabetes, found Urine Protein - Creatinine ratio more accurate than 24 hours urine protein measurement.

FIGURE 4 : APPROACH TO PATIENTS WITH PROTEINURIA [25]



Stages of Chronic Kidney Disease (CKD) as per National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines

STAGE	DESCRIPTION	GFR
1	Kidney damage with normal or high GFR	≥ 90
2	Kidney damage with mild decrease in GFR	60 – 89
3	Moderate	30 – 59
4	Severe	15 – 29
5	Kidney failure (End stage renal disease)	< 15 (or dialysis)

TABLE 2 : FURTHER INVESTIGATIONS IN PROTEINURIA [28]

Sl.No	Investigation	Inference
1	ANA	in SLE and other connective tissue diseases
2	ASO titer	Raised in post streptococcal Glomerulo nephritis
3	C3, C4	decreased in glomerulonephritis
4	ESR	Normal value helpful in ruling out inflammatory and infectious causes
5	FBS & HbA1c	Elevated in diabetes mellitus
6	Hb, PCV	Low in CKD

7	HIV, VDRL and Hepatitis serology	Human immunodeficiency virus, Syphilis, Hepatitis B & C can lead to glomerular proteinuria
8	Serum Electrolytes	Helpful in diagnosing etiology and complications
9	Lipid profile	Increased in nephrotic syndrome
10	Serum albumin	decreased in nephrotic syndrome
11	Chest Radiograph	may provide evidence of systemic disease (e.g., sarcoidosis)
12	Serum urate	Urinary tract calculi, tubulointerstitial disease
13	Renal USG	To rule out structural renal disease
14	Serum and urine protein electrophoresis	Abnormal pattern in multiple myeloma

INVESTIGATIONS DEPENDING ON TYPE OF PROTEINURIA

Glomerular proteinuria:

- Serum immunoelectrophoresis
- Urine immunoelectrophoresis
- Complements C3, C4,
- Renal biopsy
- ANA
- VDRL
- HIV/HBV/HCV serology

Tubular proteinuria:

- B2 Miroglobulin/Albumin excretion ratio
- Urinary electrophoresis
- Heavy metal screening

Over flow Proteinuria:

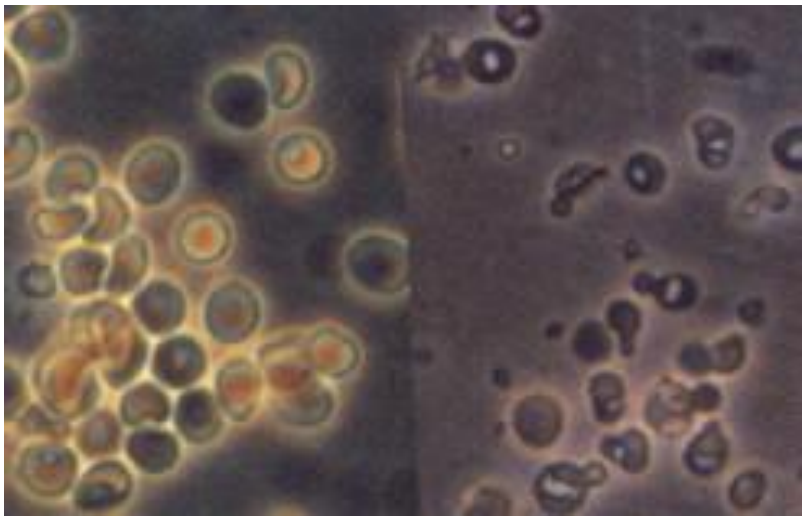
- Serum / urine electrophoresis
- Urinary light chains
- Urinary spectrophotometry

TABLE 3 : INTERPRETATION OF FINDINGS OF MICROSCOPIC EXAMINATION OF URINE [57]

No:	Microscopic finding	Pathological process
1	Fatty casts, free fat or oval fat bodies	Nephrotic syndrome
2	Leukocytes, Leukocyte casts with bacteria	Urinary tract infection
3	Leukocytes, Leukocyte casts without bacteria	Renal interstitial disease
4	Normal shaped erythrocytes	Lower urinary tract lesion
5	Dysmorphic erythrocytes	Upper urinary tract lesion
6	Erythrocytes casts	Glomerular disease
7	Waxy, granular or cellular casts	Advanced chronic renal disease
8	Eosinophiluria	drug induced acute interstitial nephritis
9	Hyaline Casts	Not associated with proteinuria; Various physiologic states, such as strenuous exercise or dehydration

URINE MICROSCOPY

A **B**

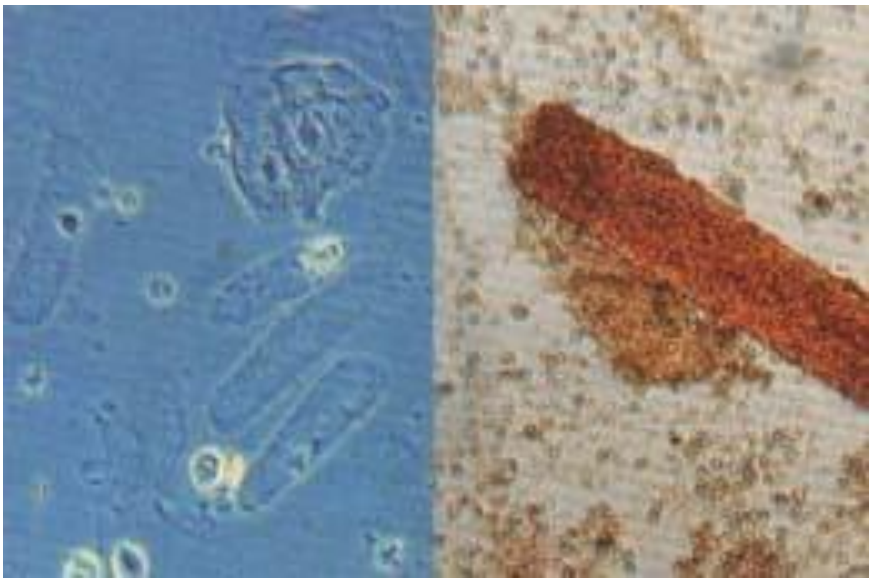


Phase contrast image of red blood cells showing :

A- Glomerular bleeding with many dysmorphic forms including acanthocytes (Teardrop forms)

B- Bleeding from lower in the urinary tract

C **D**



Phase contrast images showing:

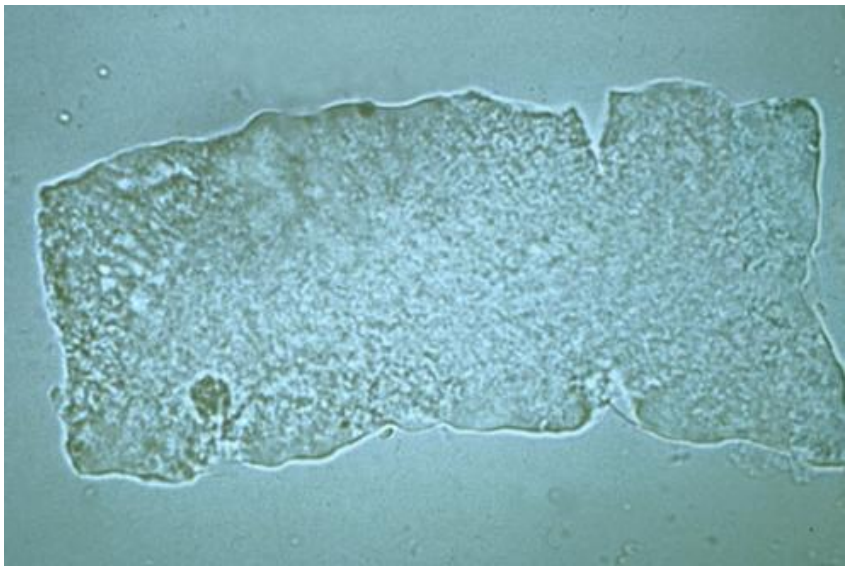
C-Hyalin casts (Normal feature of urine).

D-Numerous red cells and a large red cell cast in acute glomerular inflammation.

E

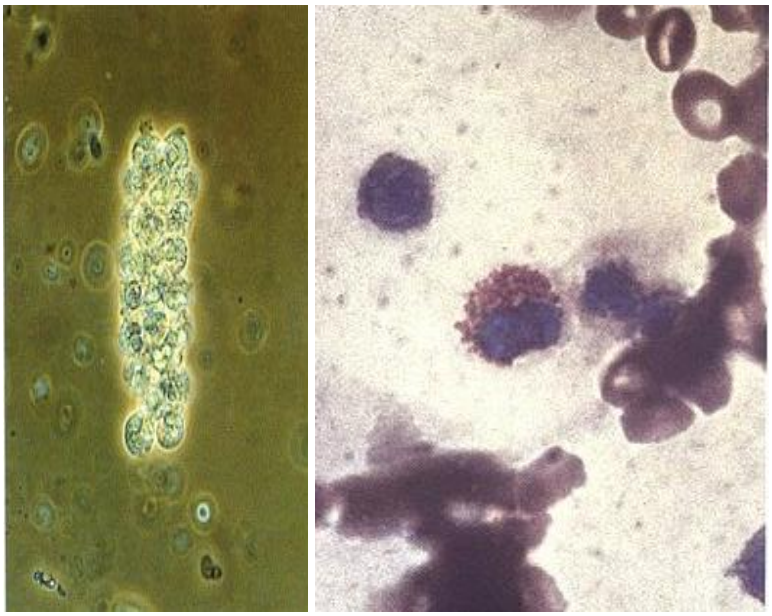
E : Fatty cast & Oval fat body

F



F : Waxy cast

G H



G : Leukocyte cast H : Eosinophiluria

I

I : Granular cast

INFLUENCE OF REDUCING PROTEINURIA ON RENAL OUTCOME

Proteinuria is an independent risk factor for progression of renal disease. A number of smaller studies in non diabetic patients suggested that the magnitude of reduction in urinary protein excretion independently predicted the magnitude in the preservation of renal function [58]. However it was not until the past decade that larger prospective trails provided support for this observation.

In APRI trial, in which patients were treated with Benazapril, the greatest reduction in risk for the development of a renal end-point was seen in patients with the greatest degree of proteinuria [59].

In MDRD study, reduction in blood pressure to levels of approximately 125/75 mm Hg in conjunction with a low-protein diet in patients with greater degrees of proteinuria (>1gm/24 hours) was associated with a slow rate of loss of GFR [60].

REIN trial demonstrated that ramipril reduced the relative risk of developing end stage renal failure by five fold and decreased the risk of massive proteinuria [61].

In another study with ACE inhibitors they found that benefit of therapy with ACE inhibitors was time dependent, with greater benefits being observed in patients treated for longer periods [62].Thus the above studies clearly shows that drugs and dietary intervention will be needed to reduce the risk of proteinuria.

MATERIALS AND METHODS

Setting	**:**	In-patients, Department of Medicine and Nephrology Thanjavur Medical College Hospital, Thanjavur.
Ethical committee Approval	**:**	obtained.
Design of study	**:**	Single center, prospective observational study.
Period of study	**:**	April -2012 to November -2012
Sample size	**:**	68 patients.

SELECTION OF STUDY SUBJECTS

INCLUSION CRITERIA:

1. Patient of either sex
2. Patient of age 13 or above
3. Nephrotic syndrome
4. Diabetic nephropathy
5. Systemic lupus erythematosus / Mixed connective tissue disease
6. Chronic kidney disease

EXCLUSION CRITERIA:

1. Patients of age less than 13 years
2. Gross hematuria
3. Females during their menstruation period
4. Patients with febrile illness
5. Dehydration
6. Intense activity
7. Cardiac failure

METHODOLOGY

A Study of 68 patients satisfying the inclusion and exclusion criteria managed by Medicine and Nephrology departments of Thanjavur Medical college were recruited for this study. The study was undertaken for eight months, from April 2012 to November 2012.

The baseline data of patients including age, sex and bodyweight were acquired. Informed consent was taken from all the participants. A detail history of the illness, general physical examination and systemic examination was done. Clinical presentation, medical illness, physical examination finding, baseline laboratory investigations, 24 hours urine protein excretion, 24 hours urine creatinine and spot urine sample protein – Creatinine ratio were estimated and were noted down in the proforma.

For the estimation of 24 hours urine proteins, these patients were provided with plastic can (5 liters capacity) with preservative (acetic acid) to collect their 24 hours urine. The time was noted and patient was advised to collect their entire 24 hours urine (excluding the first morning urine sample on the first day) in the can provided to them,

including the last void urine at the end of 24 hours. These patients were also advised to collect an untimed urine sample on the next day after the 24 hours urine collection. Patients were advised to avoid intense physical activity. No specific advice was given about water intake or dietary protein intake.

The urine for 24 hours protein and spot protein concentration was estimated by using dye binding technique with **Pyrogallol red** in auto analyzer (BTS – 350).

24 hours urine creatinine and Spot Urine Creatinine was estimated by using **modified Jaffe's method** in auto analyzer (BTS – 350).

All analyses were performed with strict adherence to standard operating procedures.

Expected minimum creatinine excretion per day was predicted as 18 mg/kg/day for males and 15 mg/kg/day for females. The adequacy of 24 hour urine collection was assessed by comparing the 24 hour urine creatinine with the expected creatinine. If the expected creatinine is similar to to what has been measured in 24 hour urine sample, the

collection was taken as adequate. Sample with incomplete collection was discarded and the participant was advised to recollect in a proper way.

Spot Urine Protein – Creatinine ratio was calculated by dividing the spot urine protein concentration by the spot urine creatinine concentration (both expressed in mg/dl).

$$\text{Protein} - \text{creatinine ratio} = \frac{\text{urine protein (mg/dl)}}{\text{urine creatinine (mg/dl)}}$$

Urine samples were evaluated by sulphosalicylic acid test for albumin, Benedict's test for sugar and by microscopy for deposits. Serum biochemical analysis was done using auto analyzer (BTS – 350) and complete blood count by using SYSMEX KX – 21 cell counter.

Patients were divided into 2 groups depending on Creatinine clearance. Creatinine clearance was estimated from age, sex, weight and serum Creatinine of the individual by the **COCKCROFT – GAULT** [63] formula given below :

$$\text{Creatinine Clearance (male)} = \frac{[140\text{-Age (yrs)}] \times \text{Wt (kg)}}{\text{serum creatinine} \times 72}$$

$$\text{Creatinine clearance (female)} = \frac{[140\text{-Age (yrs)}] \times \text{Wt (kg)} \times 0.85}{\text{serum creatinine} \times 72}$$

Group I:

Calculated Creatinine clearance > 15 ml/min

Group II:

Calculated Creatinine Clearance < 15ml/min

Each group was further divided into two sub groups depending on degree of proteinuria :

Group I A:

Calculated Creatinine clearance > 15ml/min and Nephrotic range proteinuria (> 3.5 gm/day).

Group I B:

Calculated Creatinine clearance > 15ml/min and Non Nephrotic range proteinuria (< 3.5 gm/day).

Group II A:

Calculated Creatinine clearance < 15ml/min and Nephrotic range proteinuria (> 3.5 gm/day)

Group II B:

Calculated Creatinine clearance < 15 ml/min and Non Nephrotic range proteinuria (< 3.5 gm/day)

Statistical data was analyzed using SPSS (Statistical Package for Social Service) computer program. The statistical tests used were

1. **Student T test**
2. **Paired sample correlation test**

RESULTS

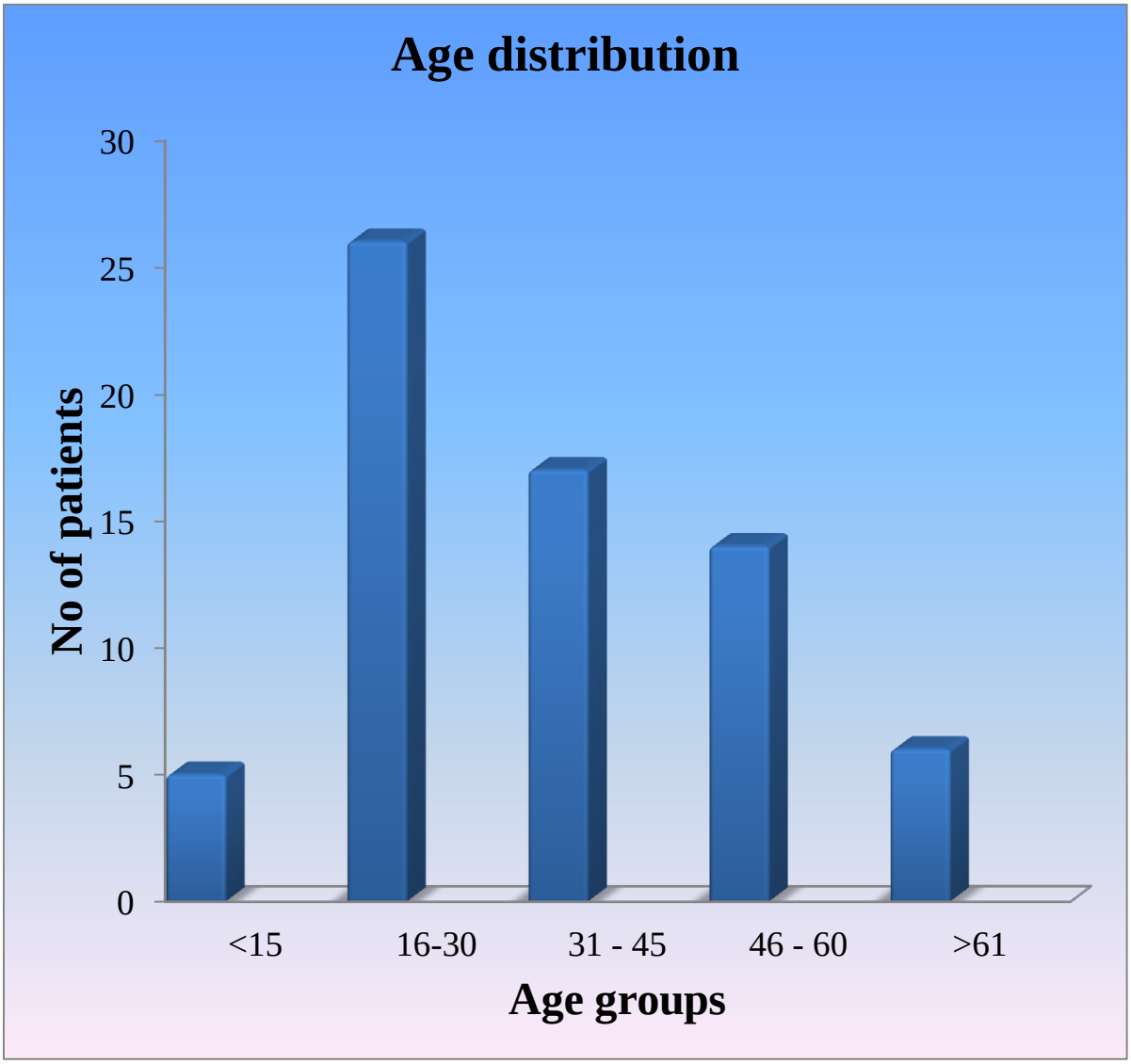
This study included 68 patients, who had proteinuria with varying degree of renal dysfunction, admitted in Medicine and Nephrology wards of Thanjavur Medical College Hospital. The patients were segregated into four groups depending on calculated creatinine clearance and degree of proteinuria. The results were tabulated and analyzed.

Total number of patients = 68

AGE DISTRIBUTION OF PATIENTS WITH PROTEINURIA :

Sl.no	Particulars	No.of respondents (n=68)	Percentage (100%)
1	13 - 15yrs	5	7.4
2	16 to 30yrs	26	38.2
3	31 to 45yrs	17	25.0
4	46 to 60yrs	14	20.6
5	61yrs & above	6	8.8

AGE DISTRIBUTION OF PATIENTS WITH PROTEINURIA



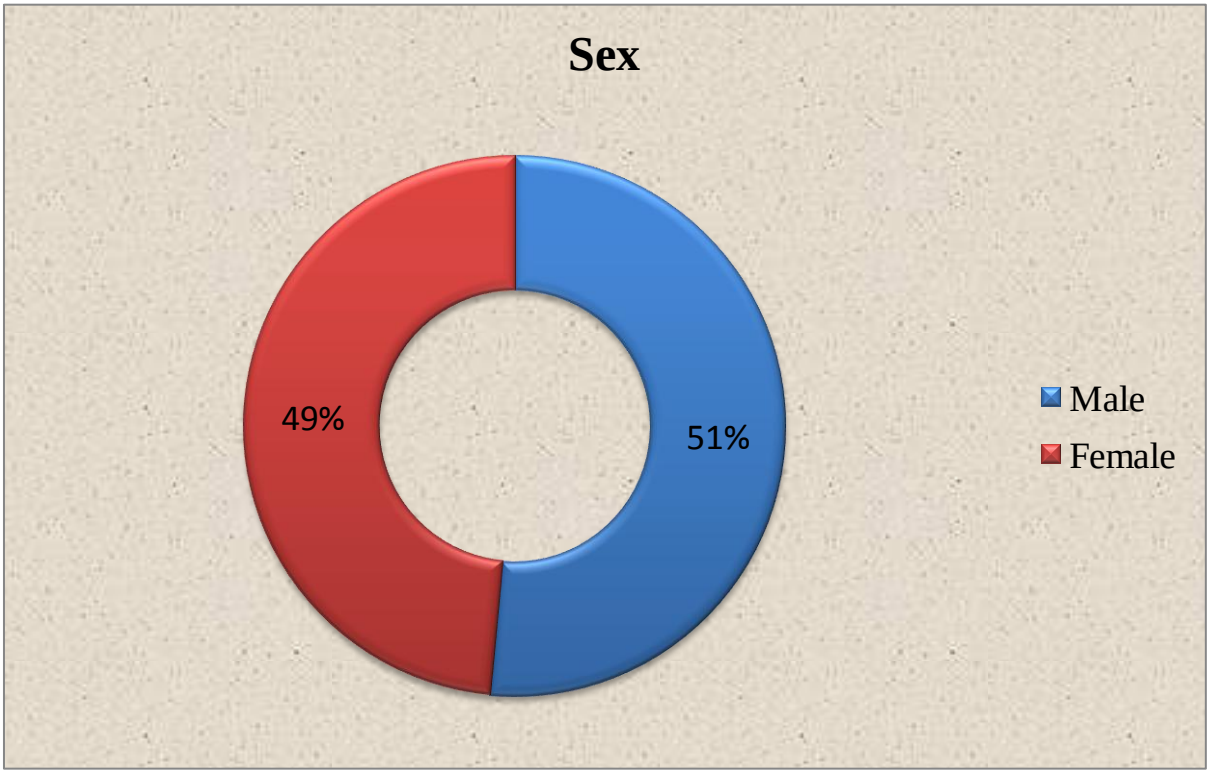
In this study the age ranged from 13 to 70 years. The incidence of proteinuria was maximum in the age group of 16 - 30 years (38.2 %) .

SEX DISTRIBUTION IN PATIENTS WITH PROTEINURIA

Sl.no	Sex	No.of respondents (n=68)	Percentage (100%)
1	Male	35	51.5
2	Female	33	48.5

In this study, number of males with proteinuria was slightly higher than that of females. The ratio of males to females with proteinuria was 1.06 : 1.

SEX DISTRIBUTION IN PATIENTS WITH PROTEINURIA

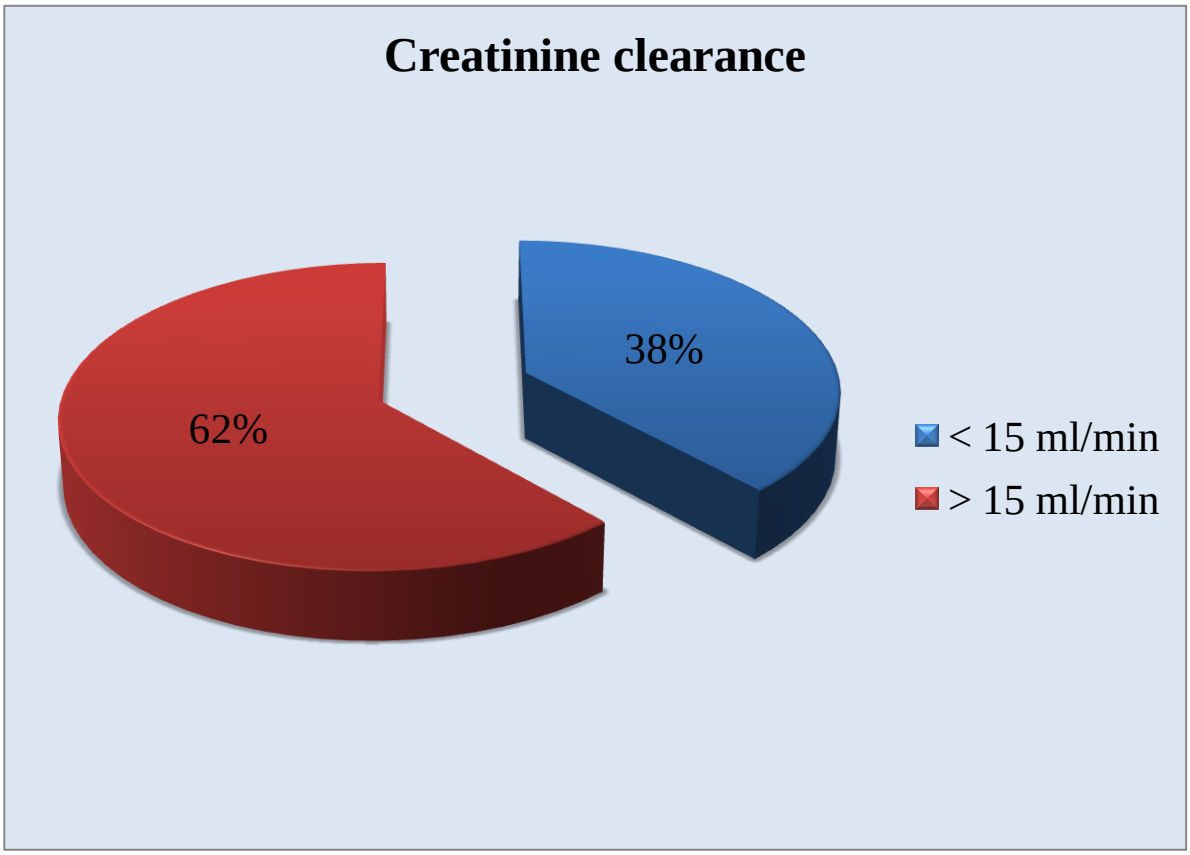


CALCULATED CREATININE CLEARANCE IN PATIENTS WITH PROTEINURIA

Sl.no	Creatinine clearance	No.of respondents (n=68)	Percentage (100%)
1	< 15 ml/min	26	38.2
2	> 15 ml/min	42	61.8

In this study, majority of patients had > 15 ml/min calculated Creatinine clearance. 42 patients (61.8 %) had a Creatinine clearance > 15ml/min and 26 patients (38.2 %) had Creatinine clearance of < 15 ml/min.

CALCULATED CREATININE CLEARANCE IN PATIENTS WITH PROTEINURIA

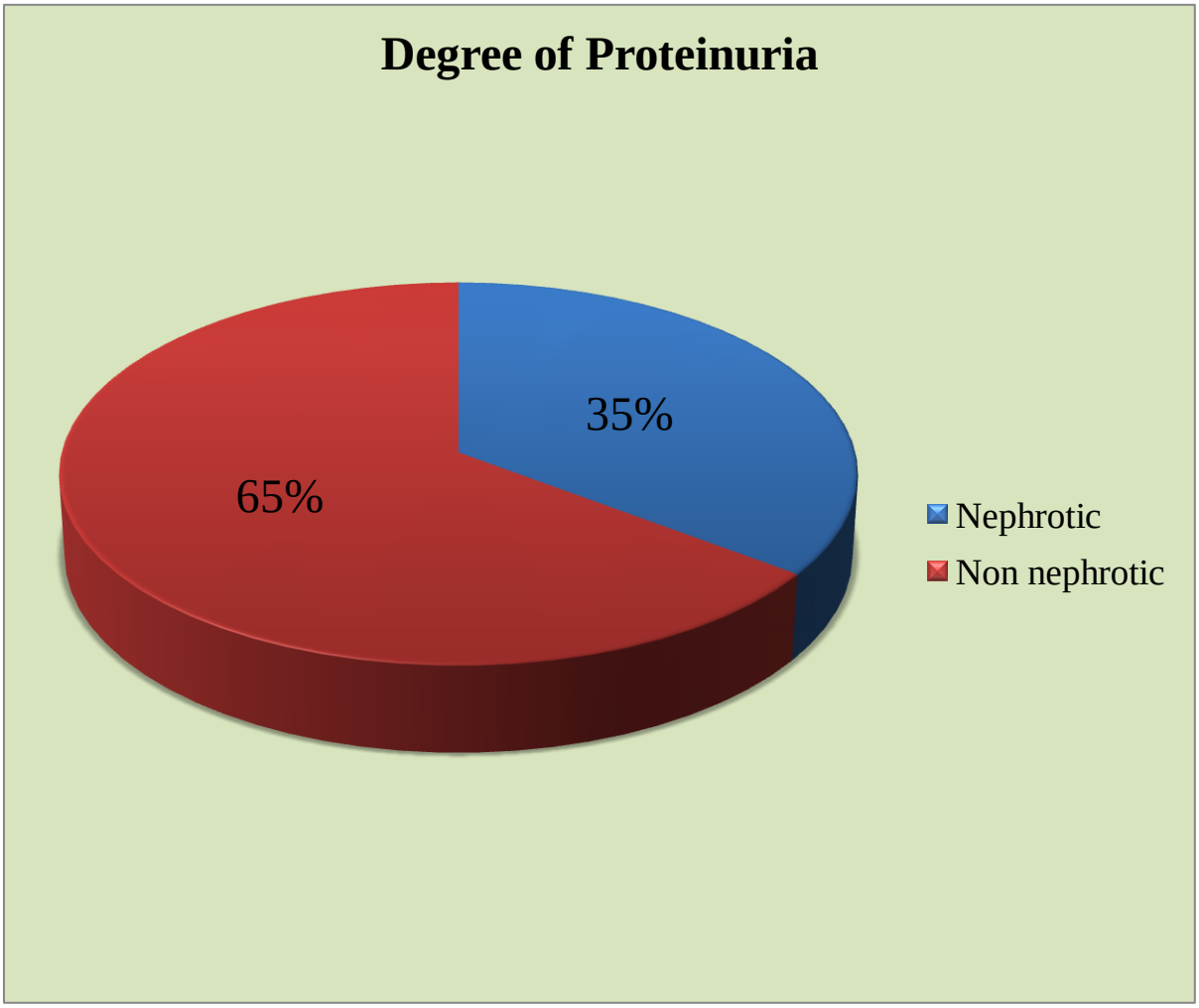


CLASSIFICATION OF PATIENTS BASED ON DEGREE PROTEINURIA

Sl.no	Proteinuria	No.of respondents (n=68)	Percentage (100%)
1	Non nephrotic (< 3.5 g/day)	44	64.7
2	Nephrotic (> 3.5 g/day)	24	35.3

Out of the 68 patients with proteinuria, 24 patients (35.3 %) had proteinuria of more than 3.5 gms/24 hours and 44 patients (64.7%) had proteinuria less than 3.5 gms/24 hours.

CLASSIFICATION OF PATIENTS BASED ON DEGREE OF PROTEINURIA



SEGREGATION OF PATIENTS INTO DIFFERENT GROUPS

The patients included in this study were segregated into 4 groups depending on degree of proteinuria and renal function

Group I A:

Chronic Kidney Disease other than End Stage Renal Disease i.e., CKD stage 1 to 4 (Calculated Creatinine clearance > 15 ml/min) with nephrotic range proteinuria (>3.5 gm/day)

Group I B:

Chronic Kidney Disease other than End Stage Renal Disease i.e., CKD stage 1 to 4 (Calculated Creatinine clearance > 15 ml/min) with non nephrotic range proteinuria (< 3.5 gm/day)

Group II A:

End stage renal disease / CKD stage 5 (Calculated Creatinine clearance < 15ml/min) with nephrotic range proteinuria (> 3.5 gm/day)

Group II B :

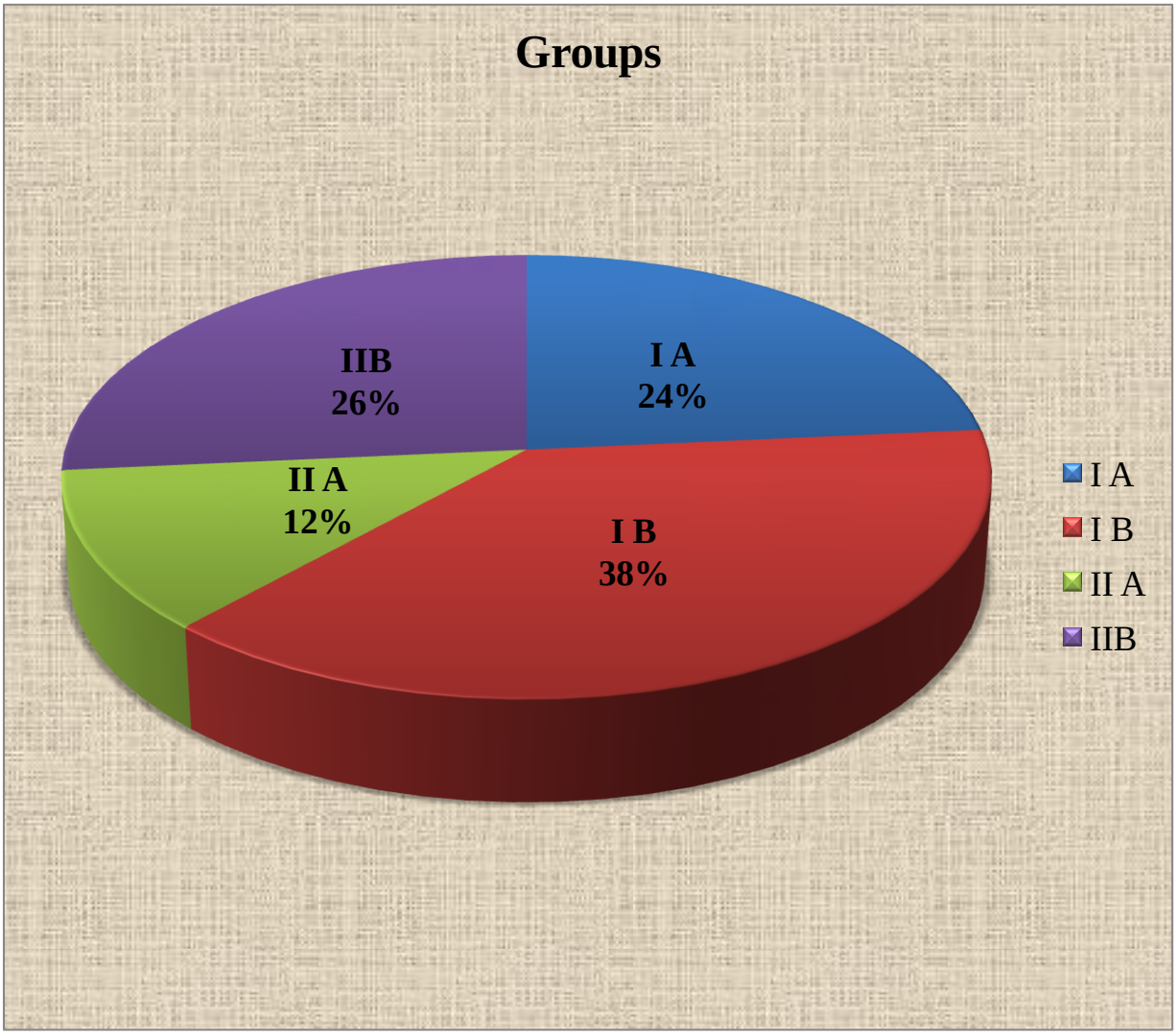
End stage renal disease / CKD stage 5 (Calculated Creatinine clearance < 15ml/min) with non nephrotic range proteinuria (< 3.5 gm/day)

Calculated Creatinine clearance	**Nephrotic proteinuria**	**Non nephrotic proteinuria**
> 15 ml/min	16	26
< 15 ml/min	8	18

Group	No.of respondents (n=68)	Percentage (100%)
I A	16	23.5
I B	26	38.2
II A	8	11.8
II B	18	26.5

In this study 16 patients (23.5%) had CKD stage 1 to 4 with nephrotic range proteinuria. There were 26 patients (38.2 %) with CKD stage 1 to 4 and non nephrotic range proteinuria. 8 patients (11.8 %) had ESRD / CKD stage 5 with nephrotic range proteinuria and 18 patients (26.5 %) had ESRD / CKD stage 5 with non nephrotic range proteinuria.

SEGREGATION OF PATIENTS INTO DIFFERENT GROUPS



STATISTICAL ANALYSIS

T-Test

Paired Samples Statistics

Correlation between Spot Urine protein creatinine ratio of the respondents and their Estimated 24 hours urine protein (gms)

Pair	Mean	N	S.D	Correlation	Sig.	Mean	S.D	t	df	Sig.
Spot Urine protein creatinine ratio	2.5759	68	1.26978	**.975**	**.000<0.05 Significant**	-.5935	.34466	-14.201	67	.000<0.05 Significant
Estimated 24 hours urine protein (gms)	3.1694	68	1.43400							

Statistical test: *Paired sample 't' test was used the above table*

Inference:

The above table indicates that there is a significant correlation between Spot Urine protein creatinine ratio of the respondents and their Estimated 24 hrs urine protein (gms). Hence the calculated value less than table value *($p<0.05$)*.

Correlation between Spot Urine protein creatinine ratio of <u>Group I A</u> and their Estimated 24 hours urine protein (gms)

T-Test - IA

Paired Samples Statistics

Pair	Mean	N	S.D	Correlation	Sig.	Mean	S.D	t	df	Sig.
Spot Urine protein creatinine ratio	4.2275	16	.39044	**.999**	**.000<0.05 Significant**	-.5263	.01586	-132.690	15	.000<0.05 Significant
Estimated 24 hrs urine protein (gms)	4.7538	16	.39789							

Statistical test: *Paired sample 't' test was used for the above table*

Inference:

The above table indicates that, there is a significant correlation between Spot Urine protein creatinine ratio of Group I A respondents and their Estimated 24 hours urine protein (gms). Hence the calculated value less than table value *(p<0.05)*.

Correlation between Spot Urine protein creatinine ratio of <u>Group I B</u> and their Estimated 24 hours urine protein (gms)

T-Test - IB

Paired Samples Statistics

Pair	Mean	N	S.D	Correlation	Sig.	Mean	S.D	t	df	Sig.
Spot Urine protein creatinine ratio	1.6908	26	.89334	**.999**	**.000<0.05 Significant**	-.2669	.09616	-14.154	25	.000<0.05 Significant
Estimated 24 hrs urine protein (gms)	1.9577	26	.97993							

Statistical test: *Paired sample 't' test was used for the above table*

Inference:

The above table indicates that there is a significant correlation between Spot Urine protein creatinine ratio of Group I B respondents and their Estimated 24 hours urine protein (gms). Hence the calculated value less than table value *(p<0.05)*.

Correlation between Spot Urine protein creatinine ratio of <u>Group II A</u> and their Estimated 24 hours urine protein (gms)

T-Test – II A

Paired Samples Statistics

Pair	Mean	N	S.D	Correlation	Sig.	Mean	S.D	t	df	Sig.
Spot Urine protein creatinine ratio	3.7450	8	.37413	**.868**	**.005<0.05 Significant**	-1.2550	.22475	-15.794	7	.000<0.05 Significant
Estimated 24 hrs urine protein (gms)	5.0000	8	.45170							

Statistical test: *Paired sample 't' test was used for the above table*

Inference:

The above table indicates that there is a significant correlation between Spot Urine protein creatinine ratio of Group II A respondents and their Estimated 24 hours urine protein (gms). Hence the calculated value less than table value *(p<0.05)*.

Correlation between Spot Urine protein creatinine ratio of <u>Group II B</u> and their Estimated 24 hours urine protein (gms)

T-Test – II B

Paired Samples Statistics

Pair	Mean	N	S.D	Correlation	Sig.	Mean	S.D	t	df	Sig.
Spot Urine protein creatinine ratio	1.8667	18	.21780	**.995**	**.000< 0.05 Significant**	-.8311	.02166	-162.772	17	.000<0.05 Significant
Estimated 24 hrs urine protein (gms)	2.6978	18	.21913							

Statistical test: *Paired sample 't' test was used for the above table*

Inference:

The above table indicates that there is a significant correlation between Spot Urine protein creatinine ratio of Group II B respondents and their Estimated 24 hours urine protein (gms). Hence the calculated value less than table value *(p<0.05)*.

Student T-Test : Sex and inference

Sex	Mean	S.D	Statistical inference
B. Urea (mg/dl)			
Male (n=35)	59.63	27.596	T=2.623 .011<0.05 Significant
Female (n=33)	43.24	23.618	
S.Creatinine (mg/dl)			
Male (n=35)	4.6771	2.53250	T=2.921 .005<0.05 Significant
Female (n=33)	2.9545	2.31747	
Creatinine clearance (ml/min)			
Male (n=35)	23.8809	17.01061	T=-1.800 .077>0.05 Not Significant
Female (n=33)	30.6709	13.83219	
Spot Urine protein creatinine ratio			
Male (n=35)	2.4946	1.24723	T=-.541 .590>0.05 Not Significant
Female (n=33)	2.6621	1.30697	
Estimated 24 hrs urine protein (gms)			
Male (n=35)	3.1460	1.37428	T=-.138 .891>0.05 Not Significant
Female (n=33)	3.1942	1.51577	

Df=66

Statistical test: *Student't' test was used the above table*

Inference:

The above table denotes that there is a significant correlation between sex of the respondents and their B. Urea (mg/dl), S.Creatinine (mg/dl). Hence the calculate value less than table value *(p<0.05)*.

The above table signifies that there is no significant correlation between sex of the respondents and their Creatinine clearance (ml/min), Spot Urine protein creatinine ratio, Predicted 24 hrs urine protein, (gms), Estimated 24 hrs urine protein (gms). Hence the calculate value greater than table value *(p>0.05)*.

So both spot urine protein creatinine ratio and 24 hour urine protein tests can be performed in both sexes with accuracy.

Oneway ANOVA f test : Groups & Inference

Outcome	Mean	S.D	SS	Df	MS	Statistical inference
B. Urea (mg/dl)						
Between Groups			36514.821	3	12171.607	F=66.082 .000<0.05 Significant
I A (n=16)	30.44	7.220				
I B (n=26)	35.42	7.900				
II A (n=8)	83.50	15.856				
II B (n=18)	79.89	21.263				
Within Groups			11788.061	64	184.188	
S.Creatinine (mg/dl)						
Between Groups			359.125	3	119.708	F=94.352 .000<0.05 Significant
I A (n=16)	1.6188	.43393				
I B (n=26)	2.3077	.98343				
II A (n=8)	6.6375	1.60351				
II B (n=18)	6.7889	1.45921				
Within Groups			81.199	64	1.269	
Creatinine clearance (ml/min)						
Between Groups			12431.668	3	4143.889	F=61.501 .000<0.05 Significant
I A (n=16)	43.7156	10.58440				
I B (n=26)	33.4442	9.92762				
II A (n=8)	10.5038	2.51654				
II B (n=18)	10.8300	2.69573				

Within Groups			4312.253	64	67.379	
Spot Urine protein creatinine ratio						
Between Groups			84.003	3	28.001	F=74.593 .000<0.05 Significant
I A (n=16)	4.2275	.39044				
I B (n=26)	1.6908	.89334				
II A (n=8)	3.7450	.37413				
II B (n=18)	1.8667	.21780				
Within Groups			24.024	64	.375	
Estimated 24 hrs urine protein (gms)						
Between Groups			109.149	3	36.383	F=81.343 .000<0.05 Significant
I A (n=16)	4.7538	.39789				
I B (n=26)	1.9577	.97993				
II A (n=8)	5.0000	.45170				
II B (n=18)	2.6978	.21913				
Within Groups			28.626	64	.447	

Statistical test: *Oneway ANOVA 'f' test was used the above table*

Inference:

The above table denotes that there is a significant correlation among the groups

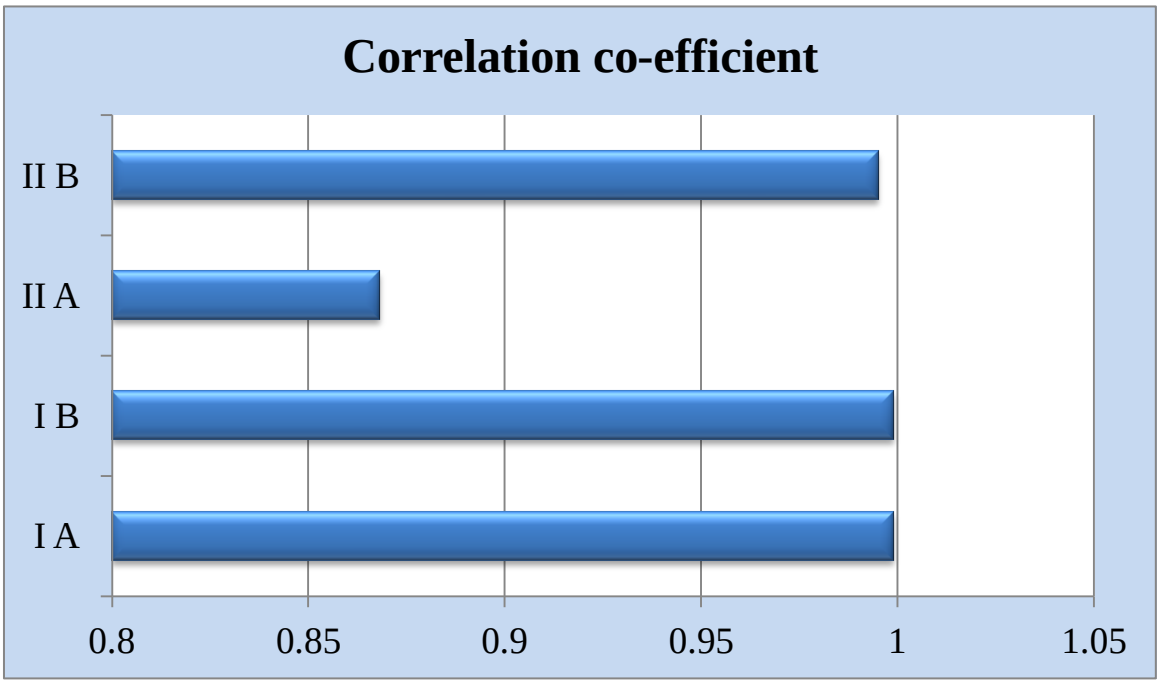
between outcome of the respondents and their B. Urea (mg/dl), S.Creatinine (mg/dl), Creatinine clearance (ml/min), Spot Urine protein creatinine ratio, Predicted 24 hrs urine protein (gms), Estimated 24 hrs urine protein (gms). Hence the calculate value less than table value *(p<0.05)*.

CORRELATION CO–EFFICIENT

Groups	Correlation co-efficient (r)
I A	**0.999**
I B	**0.999**
II A	**0.868**
II B	**0.995**

In the four groups studied, there was good correlation (0.975) between spot urine protein creatinine ratio and 24 hours urine protein. The correlation was best in patients with Creatinine Clearance > 15 ml/min (with both nephrotic and non nephrotic range proteinuria) (r=0.999). The correlation was least in patient with End stage renal disease and nephrotic range proteinuria (r=0.868).

CORRELATION CO-EFFICIENT IN DIFFERENT GROUPS



DISCUSSION

Measurement of urinary proteins over 24 hours is the definitive method to quantify proteinuria. However, continued collections of urine are cumbersome and often fallacious due to frequent collection errors [47].

Sharma et al [64] used 3 hours urine collection to estimate daily urinary protein excretion in normal Indian subjects. However this method is unsuitable for those with severe oliguria and for the pediatric age group. Apart from this, minute fallacy in the duration of collection period or missing a sample of urine would be exaggerated several folds when the values are interpreted in terms of 24 hours proteinuria.

To overcome these difficulties, we estimated 24 hour urinary protein excretion from single voided, spot urine sample.

The randomly obtained urine protein Creatinine ratio can be expected to predict 24hour protein for several reasons [46]. First, the amount of protein and Creatinine in the urine are determined by their excretion rates and by the tubular reabsorption of water. Since water

reabsorption is the same for both values of the same specimen, the protein – creatinine ratio therefore reflects the excretion rate of protein relative to creatinine. Second, when both urinary protein and urine creatinine values are reported in similar units (mg/dl), The Protein Creatinine ratio can be thought of as the excretion rate of urinary protein in grams relative to the excretion of 1 gm creatinine. Finally, since the average person excretes approximately 1 gm/day creatinine, the ratio there for can be directly used to estimate 24 hour urinary protein in grams/day.

In this study most of the patients (98.5 %) who had 24 hour proteinuria > 3.5 gms had Protein Creatinine ratio of > 3.5 gms in spot urine.

Vijay et al[69] **and Mohan et al** [70] in their study found that prevalence of diabetes related proteinuria was 18.7% and 9.4 % respectively in patients with type 2 DM. In this study 19.1 % of patients with proteinuria were diabetics.

Ali A et al [70]**, Dilshad Ahmed Khan et al** [71]**, Satish Basanagouda Biradar et al** [72] **and Rathi et al** [68] reported good correlation between daily urinary protein excretion rate and PCR from a spot urine sample.

Ginsberg et al [48] also, however pointed out the significance of concurrent rate of urinary creatinine excretion in estimating 24hour proteinuria.

Ruggenenti et al[56] in his studies concluded that the 24 hours urine protein can be directly predicted from a random urine specimen by estimating Protein-Creatinine ratio.

Shaw et al[71] in assessment of proteinuria from protein creatinine index found good correlation between protein-Creatinine ratio and 24 hour urine protein.

In this study, the protein - Creatinine ratio of randomly obtained urine specimen correlated well (r = 0.975) with 24 hours urine protein with varying degree of proteinuria and normal to severely impaired renal functions. This is similar to the previous studies by **Ginsberg et al** (0.97), **Houser et al** (0.98), **Schwab et al** (0.98), **Lemann and Doumas et al** (0.97), **Combs et al** (0.98), **Mitchell et al** (0.98), **Chitalia et al** (0.97) and **Alfredo et al** (0.98) [73].

Mohan et al [67] studied the correlation between the expected 24 hours urine protein calculated from spot urine protein – cCreatinine ratio and the estimated 24 hours urine protein in type 2 DM. The positive correlation was good, but was less with increasing degree of proteinuria. Correlation co efficient (r) values were 0.96, 0.86, 0.74 in groups of patients with proteinuria < 200 mg /day, 201 – 999 mgs/day and more than 1 gm/day respectively.

Sharma et al [64] studied the correlation between the protein creatinine ratio in spot urine sample with 24hours urine protein with varying degree of renal dysfunction and concluded a good positive correlation in patients with advanced renal failure. Correlation co efficient (r) values were 0.889, 0.788, 0.375 in patients with serum creatinine < 1.5 mg/dl, 1.5-4 mg/dl, > 4 mg/dl respectively.

Siwach et al [65] found that the product of protein creatinine ratio and estimated daily urinary creatinine excretion positively correlated well with the estimated 24 hours urine protein in patient with normal or mild to moderately impaired renal function (r = 0.88 and 0.99), but poorly correlated in patients with advanced renal failure (r = 0.56)

Xing G et al [74] found out that protein creatinine ratio can be used as an alternative for 24 hours urine protein estimation in patients with creatinine clearance > 10 ml/min.

Goldman R et al [66] found that the possible reason for poor correlation in these patients is that with progression of renal failure the urinary Creatinine excretion falls especially after serum creatinine exceeds 6 mg/dl .

In this study, the best positive correlation was in patients with creatinine clearance > 15 ml/min (with both nephrotic and non nephrotic range proteinuria) (r=0.999). The correlation was least in patients with end stage renal disease and nephrotic range proteinuria (r=0.868).

Vishwanathan et al [72]in his study showed that estimated proteinuria calculated from urinary Protein- Creatinine ratio in a random urine sample is useful in successive evaluation of kidney function on a follow up basis.

LIMITATIONS OF THIS STUDY :

1. Natural improvement of the study group
2. Lab error
3. Patient may be on drugs
4. Small group; further large studies may be needed
5. Correlation among spot urine samples of different timings not scrutinized.

CONCLUSION

1. Protein Creatinine ratio in the spot urine sample is found to be an useful index for quantification of proteinuria in patients with varying degree of proteinuria and renal dysfunction.

2. There was good positive correlation between spot urine Protein Creatinine ratio and 24 hours estimated protein.

3. The correlation was best in patients with normal / mild - severely impaired renal dysfunction with both nephrotic and non nephrotic range proteinuria. The positive correlation was least in patients with end stage renal disease (CKD stage 5) with nephrotic range proteinuria.

4.Urine protein creatinine ratio is easy to perform, inexpensive and less time consuming method for measuring of proteinuria. It can thus be used in the out patient setting for screening and quantification of proteinuria as an alternative to 24 hour urine protein estimation. It can be used for follow up and assessment of responsiveness to therapy, by which a physician / Nephrologist can timely intervene so that progression of kidney disease can be retarded and prognosis of cardiovascular disease in diabetes mellitus can be improved.

BIBLIOGRAPHY

1. **Cameron J S.** The Patient with Proteinuria and/or haematuria. Oxford Textbook of Clinical Nephrology 3rd edition, 2005.
2. **Denker B, Brenner B.** Azotemia and Urinary abnormalities. Harrison's Principles of Internal medicine. 17th edition, 2008.
3. **Alder S G, Fairley K.** The patient with Hematuria, Proteinuria or both, and abnormal urinary finding on urinary microscopy. Manual of Nephrology. 7th edition; 2010.
4. **Keane W F.** Proteinuria : its clinical importance and role in progressive renal disease. American Journal of Kidney Disease 2000 April, 35: S 97- S 105.
5. **Keane WF, Eknoyan G.** Proteinuria, Albuminuria, Risk, Assessment Detection, Elimination (PARADE): A position paper of National Kidney foundation. American Journal of Kidney Disease 1999; 33: 1004-1010.
6. **Carroll MF, Temte JL.** proteinuria in adults: A diagnostic approach. American family physician 2000; 62:1333-1340.
7. **Bectham R, Cattell WR.** Proteinuria :Pathophysiology, significance and recommendation for measurement in clinical practice. Ann Clin Biochem 1993; 30 : 425-434.
8. **Flanc RS, Robert MA, Strippoli GF, Chadban SJ, Kerr PG,**

Atkins RC. Treatment of diffuse proliferative lupus nephritis: A meta – analysis of randomized controlled trials. Am J Kidney Dis 2004; 43: 197-208

9. **Glassrock RJ. Postural (Orthostatic) Proteinuria** N Engl J Med 1980; 18: 395-406.
10. **Watt GF, Morris RW, Khank, Polaka** Urinary albumin exerction in health adult subjects. Clin Chim Acta 1988; 172: 191-8.
11. **Spring berg PD et al.** Fixed and reproducible orthostatic proteinuria: Results of a 20 year follow up study: Annals of internal medicine 1982; 97: 516-519.
12. **Robinson RR.** Isolated proteinuria in asymptomatic patients. Kidney International 1980; 18: 395-406.
13. **Wam LL, Yano S, Hiromurak, Tsukaday, Tomonos, Kawazer.** Effects of posture on Creatinine clearance and urinary protein exerction in patients with various renal diseases 1995.
14. **Devarajan P**. Mechanism of orthostatic proteinuria: Lessions from a transplant donor. Journal of the American society of Nephrology 1993; 4: 36-39.
15. **Shintaku N, Takahashi Y, Akaishi K, Sano A, Kuroda Y** Entrapment of the left renal vein in children with orthostatic

proteinuria. Pediatric Nephrology 1990; 4:324-327.

16. **Hotter TH.** Proteinuria Kidney 1987; 20:13.

17. **Kallmeyer G, Miller NM,** Urinary changes in ultra-long distance marathon runners. Nephron 1993; 64: 119-121.

18. **Poortmans JR, Rampaer L, Walf JC**. Renal Protein exerction after exercise in man. Eur J Apply Physiol Occup Physiol 1989; 48: 476-480.

19. **Poortms JR, Labilloy D.** The influence of work intensity on post exercise proteinuria. Eur J Appl Physiol Occup Physiol 1988; 57:260-3.

20. **Abuelo JG.** proteinuria : Diagnostic Principles and procedures. Ann Intern Med 1983:98; 186-191.

21. **McConnell KR, Bia MJ**. The Evaluation of Proteinuria: An approach for the internist. Res Staff Physician 1994; 41-48.

22. **Savin VJ**. Mechanism of proteinuria in non inflammatory glomerular disease. American Journal of Kidney Disease 1993; 21: 347-362.

23. **Daniels BS**. The role of the glomerular epithelial cell in the maintenance of the glomerular filtration barrier – American Journal of Nephrology 1993; 13: 318-323.

24. **Almeida AF**. Clinical Approach to a Patient with Renal Disease ;2003.

25. **Turner AN**, **savill** J**, Stewart LH, Camming** A . Kidney and Genitourinary Disease. Davidson's principles and practice of medicine. 21st edition 2010.

26. **Tulkensn PM**, Experimental studies on Nephrotoxicity of Aminoglycosides in low doses. Am J Med 1986; 80 (suppl 6 B) : 105-114.

27. **Schardijn GHC** statius van eps LW. Stout – zonneveld AAM et al . Urinary B2- Microglobulin in urinary tract infections, Acta Clin Belg 1980; 35 :21.

28. **Glassrock RJ.** Proteinuria. Text book of Nephrology. 3rd edition Baltimore : William and Wilkins, 1995.

29. **Longo DL, Anderson KC,** Plasma Cell Disorders Harrison's Principles of Internal medicine. 17th edition, 2008.

30. **Beinenstock J, Tomasi TB**. Secretory gamma – A in normal urine. Journal of Clinical Investigation 1968; 47: 1162-1171.

31. **Rosenman E, Boss JH** . Tissue antigens in normal and pathologic urines : A review Kidney International 1979;16: 337-344.

32. **MC Queen EG** Composition of urinary casts. Lancet 1966: 397-398.

33. **Rustecki GJ, Goldsmith C, Sehreiner G**E characterization of proteins in urinary casts. Fluorescent antibody identification of

Tanam horsfan protein in matrix and serum protein in granules. New England Journal of Medicines 1971; 284: 1049-1052.

34. **Powers AC.** Diabetes Mellitus Harrison's Principles of Internal medicine. 17th edition, 2008.

35. **Mohan V.** Chromic Complication of Diabetes Mellitus. API Textbook of Medicine. 7 edition; 2003.

36. **Messent JW, Elliot TG, Hill RG, Jarrett RG, Keen H, Viberti GC.** Prognostic Significance of Microalbuminuria in insulin dependent diabetes mellitus: A twenty – three year followup study. Kidney Int 1992; 41:836-839.

37. **Andersons S.** Proteinuria. Primer on Kidney Disease. 2nd edition Boston : National Kidney Foundation Academic Press ;1998.

38. **Frier BM, Fisher BM,** Diabetes Mellitus Davidson's principles and practice of medicine. 21st edition 2010.

40. **Davidson MB, Smiley JF.** Relationship between dipstick positive proteinuria and albumin; Creatinine ratio, J Diabetes Complication 1999; 31: 52-55.

41. **Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Pavving HH et al.** Nephrology in Diabetes. Diabetes Care 2004; 27: S 79-S 83.

42. **Meyer NL, Mercer BM, Friedman SA Sibai BM**. Urinary dipstick protein a poor predictor of absent or severe proteinuria.

AM J Obstet Gynecol 1994; 170: 137-141.

43. **John WA.** Chemical tests used in urinalysis. Hutchison's clinical methods. 21st edition London : W.B.saunders; 2002.

44. **Bahadur MM, shah SN** urinary protein in Diabetes. The Asian J of Diabetology 2001; 3; 14-16.

45. **Halsted CH**. Malnutrition and Nutritional Assessment. Harrison's Principles of Internal medicine. 16th edition. New Delhi :Mc Graw – Hill medical publishing division; 2005.

46. **Rodby RA, Rohde RD, Lewis EJ, Sharon J, Pohl MA , Bain RP et al.** The ratio as a predictor of 24 hour urine protein exerction in Type I diabetic patients with Nephropathy; American Journal of Kidney Disease 1995; 26: 904-909

47. **Kerr DN.** Normal values in Renal Medicine. Medicine 1982; 23:1047.

48. **Gisberg JM, Chang BS, Matarese RA, Garella S.** Use of single voided urine sample to estimate quantitative proteinuria. N England Journal Medicine 1983; 309: 1543-1546.

49. **Schwab SJ, Christensen RL, Dougherty K, Klahr** S. Quantitation of proteinuria by use of protein – to- Creatinine ratio in single urine samples. Arch Intern Medicine 1987; 147; 943-4.

50. **Savdan PJ, Brown MA, Farrell T, Shaw L.** Improned methods of assessing proteinuria in hypertensive pregnancy. Br J Obstet

Gynaecol 1997; 104: 1159-1164.

51. **Silink M, Cowell CT**, **Rogers S.** First Morning urinary albumin concentration is a good predictor of 24 hours urinary albumin exerction in patients with type I (insulin dependent) Diabetes. Diabetologia 1986; 29: 97-99.

52. **Koopman MG, Krediet RT, Koomen GC.** Circadian Rhythm of proteinuria: Consequences of the use of urinary protein Creatinine ratio. Nephrol Dial Transplant 1989; 4: 9-14.

53. **Wilson DM, Anderson RL**. Protein - Osmolality ratio for the quantitative assessment of proteinuria from a random urinalysis sample An J Clin Pathol 1993; 100 :419-424.

54. **Zelmanovit Z T, Gross JL, Oliveira, De Azeredo MJ.** Proteinuria is still useful for screening and diagnosis of oovert diabetic nephropathy. Diabetes Care 1998; 21: 1076-1079.

55. **Larson TS.** Evaluation of Proteinuria. Mayo Clin Proc 1994; 69:1154-8.

56. **Wapstra FH, Navis G, De Jong PE, De Zeeuw D.** Prognostic value of the short –term anti proteinuric response to ACE inhibition for predication of GFR decline in patients with non diabetic renal disease. Exp Nephrol 1996; 4: 47-52.

57. **Maschino G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M.** Effects of the Angiotensin Converting –enzyme

inhibitor Benazepril on the progression of chronic renal insufficiency. N Eng Journal Medicine 1996; 334: 939-945.

59. **Peterson JC, Adler S, Burkart JM, Greene T, Hebert LA, Hunisicker LG** et al. Modification of Diet in Renal Disease (MDRD) Study Group: Blood pressure control, Proteinuria and Progression of renal disease. Ann Intern Medicine 1995; 123: 719-733.

60. **Ruggenenti P, Perna A, Mosconi L, Matalone M, Pisoni R, Gaspari F Remuggi G:** Proteinuria predicts end stage renal failure in non diabetic chromic nephropathies. The gruppo Italiano Dei study Epidemiologici in Nefrologia (GISEN) Kidney Int 1997 63 (Suppl): S-54-S-57.

61. **Skorecki K, Green J, Brenner BA**. Chromic Renal Failure.

Harrison's Principles of Internal medicine. 17th edition, 2008.

62. **Sharma BK, Jain PK, Jindal SK**. Urinary Protein exerction in normal Indian subjects. Indian Journal Med Res 1981; 74:286.

63. **Siwach SB, Karla OP, Sharma R, Singh V, Chopra JS.** Estimation of 24 hour protein exerction from single random urine specimen. Indian Journal Med Red 1990; 92; 105-108

64. **Goldman R**. Creatinine exerction in renal failure. Proc soc exp

Biol Med 1954; 85: 446.

65. **Mohan V, Viswanathan M.** Estimation of 24 hours proteinuria: comparison of two methods. Journal Diab Ass Ind 1987; 27:73-75.

66. **Rathi DP, Bansal RC, Malhotra K K.** Spot urine test for quantitative estimation of proteinuria. J Assoc physicians India 1985: 33: 781-783.

67. **Vijay V, Snehalatha C, Ramachandran. A et al.** Prevalence of Proteinuria in non insulin dependent diabetes. J Assoc Physicians India 1994: 42; 792-794.

68. **Mohan V, Merra R, Premalatha G, Iranda**. Frequency of proteinuria in type 2 Diabetics seen at a Diabetic center in Southern India. Post Grad Med J 2000, 76:569-573.

69. **Shaw AB,Risdon P,Lewis –Jackson JD**. Protein Creatinine Index and Albustix in assessment of Proteinuria.Br Med J 1983;287:929.

70. **Ali A.** Spot urine P/R versus 24 hours urine protein at various levels of GFR. J Pak Med 2008

71. **Dilshad Ahmed K. JPMA** 55:428:2005

72. **Satish BB** a cross sectional study JRMS 2011; 16(5): 634-639

73. **BK Yadav** . Nepal Med Coll J 2010: 12(2): 100-105

74. **Xing G**. Protein to creatinine ratio in spot urine samples as a predictor of proteinuria Clin Chim Acta 2004 Dec ; 350(1-2) :35-9

PROFORMA

Serial No:

Name:

Age: Sex: Hospital No:

Address:

Chief complaints: Duration

Past History

DM

SHT

CAHD

Renal disease

Others

General Examination:

Pallor: Pedal edema:

PR: BP:

Body weight:

Systemic examination:

CVS:

RS:

P/A:

CNS:

Others:

Diagnosis:

Investigations:

Hb (g%): RBS (mg/dl):

B. Urea (mg/dl):

S. Creatinine (mg/dl):

S. Na (mEq/L): S. K (mEq/L):

Urine R/E:

Sugar:

Albumin:

Deposits:

HIV: ANA: dsDNA:

Others:

USG abdomen:

Renal Biopsy report (if already done):

24 hours urine creatinine (mg) :

Expected 24 hours urine creatinine (mg) :

Adequacy of sample collection :

Creatinine clearance (mL/min):

Spot Urine Protein Creatinine ratio:

Estimated 24 hrs urine protein (Gms):

LIST OF ABBREVIATIONS USED

ACE	–	Angiotensin converting enzyme
ANA	–	Anti nuclear antibody
APRI	–	ACE inhibitor in Progressive Renal Insufficiency
ASO	–	Anti Streptolysin O
BSA	–	Body surface area
CKD	–	Chronic kidney disease
DM	–	Diabetes mellitus
ELISA	–	Enzyme linked immunosorbent assay
ESRD	–	End Stage Renal Disease
FBS	–	Fasting blood sugar
FSGS	–	Focal segmental glomerulosclerosis
GFR	–	Glomerular filtration rate
HBV	–	Hepatitis B Virus
HCV	–	Hepatitis C virus
HIV	–	Human Immuno deficiency virus
HSP	–	Henoch Schonlein Purpura
HT	–	Hypertension
KDOQI	–	Kidney Disease Outcomes Quality Initiative
MCTD	–	Mixed Connective Tissue Disorder
MDRD	–	Modification of Diet in Renal Disease
MPGN	–	Membranoproliferative glomerulonephritis
NKF	–	National Kidney Foundation
NSAIDS	–	Non steroidal anti inflammatory drugs
PAN	–	Polyarteritis nodosa
PSGN	–	Post streptococcal glomerulonephritis
RBC	–	Red blood cells
REIN	–	Ramipril Efficacy in Nephropathy

RIA	–	Radio immune assay
SLE	–	Systemic Lupus Erythematosus
UPEP	–	Urine protein electrophoresis
USG	–	Ultra sonogram
UTI	–	Urinary tract infection
VDRL	–	Venereal disease research laboratory

MASTER CHART															
S. No.	Name	Age	Sex	IP No.	Diagnosis	Body weight (kg)	B. Urea (mg/dl)	S.Creatinine (mg/dl)	Creatinine clearance (ml/min)	Expected 24 hr urine creatinine (mg)	Estimated 24 hr urine creatinine	Sample collection adequate or not	Spot Urine protein creatinine ratio	Estimated 24 hrs urine protein (gms)	Group
1	REVATHI	30	F	1377035	NS	62	32	1.4	44.52	930	946.6	Yes	4.22	4.73	I A
2	AMUTHA	28	F	1377060	SLE	54	32	1.8	39.67	810	853.4	Yes	2.52	2.84	I B
3	SANGEETHA	17	F	1377731	SLE	42	28	1.8	33.88	630	642.5	Yes	0.32	0.44	I B
4	KAVITHA	35	F	1376125	DM/DKD/HT	52	36	2	32.23	780	802.4	Yes	0.62	0.73	I B
5	NARIYAMMAL	52	F	1378199	CKD / HT	56	102	7.8	7.46	840	872.4	Yes	2.06	2.88	II B
6	KANNAN	35	M	1378144	NS	56	32	1.6	51.04	1008	1076.8	Yes	4.01	4.52	I A
7	PRIYANKA	18	F	1379561	NS	38	30	1.6	34.21	570	576.4	Yes	3.68	4.17	I A
8	RAJKUMAR	34	M	1378565	CKD	58	94	6.8	12.56	1044	1086.8	Yes	1.82	2.64	II B
9	KAVITHA	14	F	1380151	SLE	28	30	1.8	23.14	420	428.8	Yes	2.08	2.4	I B
10	MANGALAMBAL	65	F	1380861	CKD / HT	60	86	8.2	6.48	900	924.6	Yes	1.67	2.51	II B
11	BASTIN	47	M	1381035	CKD / HT	56	132	10.4	6.96	1008	1032.7	Yes	1.6	2.42	II B
12	SELVARAJ	65	M	1381202	CKD / HT	60	108	8.6	7.27	1080	1092.4	Yes	2.2	3.01	II B
13	RAVI	27	M	1381220	CKD	50	64	5.6	14.01	900	908.2	Yes	1.98	2.76	II B
14	VEDHAVALLI	20	F	1381627	SLE	48	34	1.4	48.57	720	726.4	Yes	2.23	2.55	I B
15	VEDHA	67	M	1381776	CKD	62	52	3.8	16.54	1116	1132.6	Yes	2.04	2.35	I B
16	RADHAKRISHNAN	48	M	1382418	DM/DKD	64	78	5.8	14.1	1152	1168.4	Yes	2.02	2.87	II B
17	SAKTHIVEL	13	M	1383212	NS	28	26	1.6	30.87	504	512.8	Yes	4.23	4.76	I A
18	DURAIRAJ	40	M	1382386	SLE	54	28	1.6	46.88	972	984.6	Yes	0.21	0.33	I B
19	SAROJA	45	F	1383345	DM / CKD	56	74	6.6	9.52	840	848.4	Yes	4.08	5.39	II A
20	BHUVANESWARI	28	F	1383328	MCTD	42	26	1.4	39.67	630	642.6	Yes	0.24	0.36	I B
21	PASPEEN	42	M	1383481	DM / CKD	58	72	5.8	13.6	1044	1058.4	Yes	3.56	4.88	II A
22	RAJENDRAN	52	M	1383639	DM/DKD/HT	61	56	3.2	23.3	1098	1104.8	Yes	4.21	4.74	I A
23	VEDHAVALLI	20	F	1386634	NS/DPGN	44	32	1.5	41.56	660	672.8	Yes	4.62	5.13	I A
24	KANAKAVALLY	32	F	1387155	SLE/LN	52	36	2	33.15	780	786.4	Yes	2.03	2.36	I B
25	SATHYAMOORTHY	27	M	1386330	DM / CKD	48	84	7.8	9.66	864	878.6	Yes	3.98	5.32	II A
26	MUTHULAKSHMY	19	F	1386217	SLE	45	28	1.6	40.18	675	678.4	Yes	0.22	0.34	I B
27	KAVITHA	15	F	1387536	SLE	32	26	1.5	37.04	576	582.4	Yes	0.28	0.4	I B

MASTER CHART															
S. No.	Name	Age	Sex	IP No.	Diagnosis	Body weight (kg)	B. Urea (mg/dl)	S.Creatinine (mg/dl)	Creatinine clearance (ml/min)	Expected 24 hr urine creatinine (mg)	Estimated 24 hr urine creatinine	Sample collection adequate or not	Spot Urine protein creatinine ratio	Estimated 24 hrs urine protein (gms)	Group
28	SAMINATHAN	42	M	1387859	CKD / HT	51	78	7.2	9.64	918	934.3	Yes	1.93	2.76	II B
29	ARUMUGAM	25	M	1386690	CKD	48	67	5.8	13.22	864	878.5	Yes	1.93	2.82	II B
30	MANOHARAN	48	M	1387279	CKD	62	52	4.8	12.78	1116	1134.7	Yes	2.01	2.84	II B
31	ROJAPATHY	50	M	1384450	CKD / HT	64	64	7.2	11.11	1152	1172.3	Yes	1.42	2.24	II B
32	HARIPRIYA	14	F	1389255	NS	32	28	1.4	34	480	486.7	Yes	4.42	4.96	I A
33	LAKSHMI	39	F	1389268	CKD	54	42	3.6	17.89	810	822.5	Yes	1.44	1.64	I B
34	DHANAM	63	F	1389414	DM/CKD	60	61	3.8	14.35	900	912.8	Yes	3.06	4.42	II A
35	STEPHEN AROKIYARAJ	26	M	1389806	CKD	62	38	2.6	37.76	1116	1130.4	Yes	1.04	1.26	I B
36	KANAKAVALLY	27	F	1388772	SLE	48	32	1.6	40.02	720	726.4	Yes	1.24	1.45	I B
37	MURUGAN	23	M	1389602	NS	56	26	1.4	65	1008	1029.2	Yes	4.97	5.51	I A
38	VIGNESWARAN	14	M	1390201	NS	41	25	1.3	55.19	738	746.3	Yes	3.64	4.18	I A
39	JAYAPAL	50	M	1391084	CKD / HT	61	46	4.2	18.15	1098	1110.4	Yes	2.23	2.56	I B
40	SAROJA	65	F	1391088	DM / CKD	64	108	8.9	6.37	960	972.3	Yes	3.64	4.98	II A
41	VINO	17	F	1389400	SLE/LN	46	32	2.1	31.81	690	698.5	Yes	2.24	2.56	I B
42	AMIRTHAVALLI	24	F	1390755	FSGS	48	30	1.6	41.08	720	731.2	Yes	4.41	4.94	I A
43	ARUNACHALAM	60	M	1391453	CKD / HT	64	96	7.8	9.12	1152	1176.8	Yes	1.86	2.69	II B
44	PARVATHI	45	F	1391614	DM / CKD	52	78	5.8	10.06	780	784.5	Yes	1.73	2.56	II B
45	RADHIKA	21	F	1392681	SLE/LN	50	34	1.6	51.65	750	758.5	Yes	2.41	2.73	I B
46	ANANTHI	26	F	1391032	SLE/LN	48	34	2.1	30.76	720	734.4	Yes	2.67	3.02	I B
47	DEVARAJ	55	M	1392654	CKD / HT	64	76	5.2	14.53	1152	1174.2	Yes	1.83	2.66	II B
48	THIRUSELVAM	45	M	1393026	CKD	58	56	4.8	15.94	1044	1062.3	Yes	1.74	2.18	I B
49	PATHIMISABEEVI	32	F	1392439	NS/MEMBRANEOUS	51	26	1.6	40.64	765	774.2	Yes	4.24	4.77	I A
50	MANSOOR ALI	17	M	1392852	NS/MCN	44	26	1.4	53.69	792	804.3	Yes	4.97	5.52	I A
51	MUTHU	33	M	1393967	SLE/LN	54	34	2	40.13	972	990.3	Yes	2.56	2.88	I B
52	KARTHIK	19	M	1394438	CKD	48	68	5.8	13.91	864	879.5	Yes	1.64	2.48	II B

MASTER CHART															
S. No.	Name	Age	Sex	IP No.	Diagnosis	Body weight (kg)	B. Urea (mg/dl)	S.Creatinine (mg/dl)	Creatinine clearance (ml/min)	Expected 24 hr urine creatinine (mg)	Estimated 24 hr urine creatinine	Sample collection adequate or not	Spot Urine protein creatinine ratio	Estimated 24 hrs urine protein (gms)	Group
53	JOTHI	27	F	1E+06	NS	49	28	1.6	40.85	735	741.6	Yes	4.21	4.74	I A
54	CHINNAYYAN	60	M	1E+06	CKD / HT	64	89	7.8	9.12	1152	1167.3	Yes	2.03	2.86	II B
55	ILAYARAJA	30	M	1E+06	NS	58	30	1.6	55.38	1044	1067.5	Yes	3.93	4.46	I A
56	DHARMALINGAM	40	M	1E+06	DM/DKD	59	34	2.1	39.02	1062	1074.3	Yes	2.68	3.01	I B
57	JAYARAJAN	25	M	1E+06	CKD	54	45	4.2	20.54	972	987.4	Yes	1.78	2.02	I B
58	JAYARAMAN	55	M	1E+06	CKD / HT	58	58	6.4	10.7	1044	1056.7	Yes	1.64	2.48	II B
59	AROKIYAMARY	58	F	1E+06	SLE/LN/CKD	64	48	5.2	11.91	960	968.9	Yes	2.23	3.08	II B
60	CHITHRADEVI	42	F	1E+06	SLE/LN	54	28	1.6	39.05	810	827.4	Yes	2.24	2.58	I B
61	SAMMATHAPILLAI	70	M	1E+06	DM/CKD	58	103	7.6	10.39	1044	1050.3	Yes	4.28	5.62	II A
62	KUMARESAN	18	M	1E+06	CKD	46	46	3	25.98	828	834.4	Yes	1.42	1.74	I B
63	SHANTHI	26	F	1E+06	SLE/LN	49	32	1.8	36.64	735	744.3	Yes	2.86	3.2	I B
64	SHANTHI	35	F	1E+06	NS	54	30	1.6	41.84	810	834.4	Yes	4.02	4.56	I A
65	JOSEPHINE MARY	25	F	1E+06	SLE/LN	49	32	2	33.26	735	744.6	Yes	2.62	2.97	I B
66	VIJAYA	35	F	1E+06	NS	56	30	1.5	46.28	840	852.8	Yes	3.86	4.37	I A
67	BANUMATHY	50	F	1E+06	DM/CKD	64	88	7.2	9.44	960	974.6	Yes	3.72	5.05	II A
68	PERIYASAMY	60	M	1E+06	DM/CKD	52	78	5.4	10.7	936	948.4	Yes	3.64	4.34	II A